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# OSTEOSARCOMA

## A review of 96 cases

by

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The classification of osteosarcoma has tended in recent years to become more sharply defined with the recognition of such growths as chondrosarcoma, primary fibrosarcoma of bone and juxtacortical osteosarcoma as separate clinical and pathologic entities. The largest single group of osteosarcomas published to date is a series of 430 cases from the Mayo Clinic, covering the period 1909—1955 (COVENTRY and DAHLIN 1957). The authors reported in this group a five-year survival of 19.3 %. This figure is higher than that of many others who have had experience with this neoplasm. JAFFE (1958) and LICHTENSTEIN (1959) stated that a five-year survival rate after surgical therapy of osteogenic sarcoma is rarely over 5 to 10 %. The five-year survival rate in a Norwegian material was 10.7 % (EIDE et coll.). Certain malignant tumors have for many years in Sweden been referred to a few central tumor clinics and it has consequently been possible to collect a large group of bone tumor cases in a Bone Tumor Registry at Karolinska Sjukhuset, Stockholm. We have among the osteosarcomas 96 cases that have been followed for a minimum of 3 years and which are reviewed in this communication.

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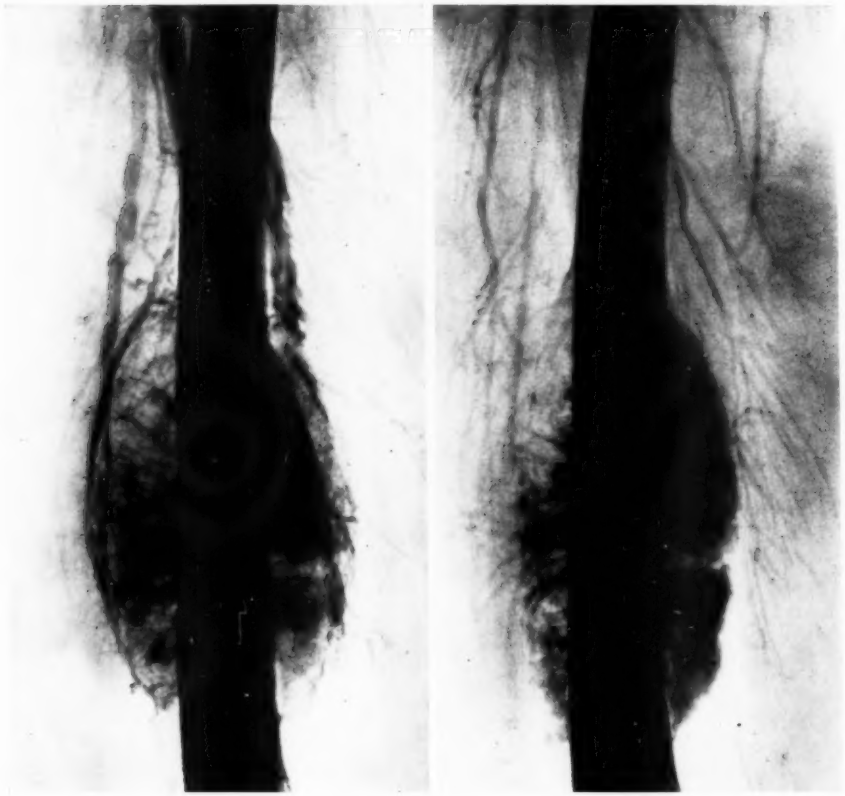

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Fig. 1. Influence of irradiation on vascularity of osteosarcoma. a) Venous phase of angiography before treatment. Many irregular vessels in tumor area. Veins draining tumor are well filled and dilated, indicating arteriovenous shunts through tumor vessels. b) Same phase of angiography after irradiation. Diminished vascularity with narrower draining veins; no obvious shunt.

*Material.* The major portion of our cases came from the three radiotherapy clinics in Stockholm, Gothenburg and Lund. A few cases from various other hospitals are included. The material does not contain all the osteosarcomas in Sweden; from that standpoint the material undoubtedly represents unintentional selection. No cases were chosen because of specific factors whether favorable or unfavorable. Tumors located in the skull, facial bones and mandible have been excluded. Only cases that had a histologically confirmed diagnosis during or before 1956, have been included. Every case was followed.

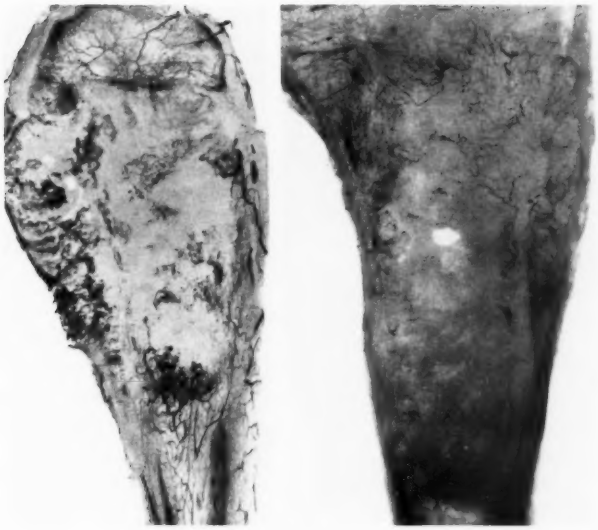

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Fig. 2. Influence of irradiation on tumor vessels. Roentgenograms of decalcified sections of injected specimens of two different chondroblastic osteosarcomas in proximal end of tibia. a) Highly vascular osteosarcoma, not irradiated. b) Another tumor after irradiation. Only sparse vessels in tumor tissue.

*Pathology.* Osteosarcoma is defined as a malignant tumor of bone with a sarcomatous stroma forming malignant osteoid and bone. Areas may be fibrous or cartilaginous, in fact these components may predominate. Tumors of bone that are purely fibrous or cartilaginous without areas of osteoid or bone formation from the malignant cells are considered as separate entities and not as previously (CADE) included as osteosarcomas. Juxtacortical osteosarcomas, osteosarcomas arising in giant cell tumors, and extra-osseous osteosarcomas have likewise been excluded from this series.

In a number of our cases the tumor was examined by angiography prior to amputation, the amputated specimen being injected with a fine barium suspension. The vasculature was studied in the whole specimen and in decalcified thin slices of the tumor (LAGERGREN et coll.). It was found that the growths were extremely vascular, the anaplastic regions being more vascular than the better differentiated areas; necrotic areas were poor in blood vessels or entirely devoid of demonstrable vessels. In several cases decreased vascularity after irradiation was shown angiographically (Fig. 1). Of special interest are two cases given preoperative irradiation therapy (one 7 000 r and the other

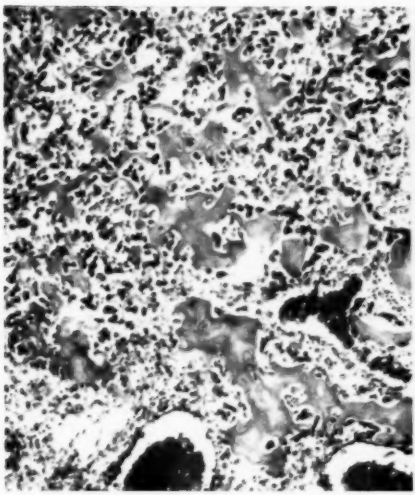

Fig. 3. Osteoblastic osteosarcoma from distal end of femur of a 19-year-old man. Patient alive 20 years after amputation. Hematoxylin-eosin. × 100.

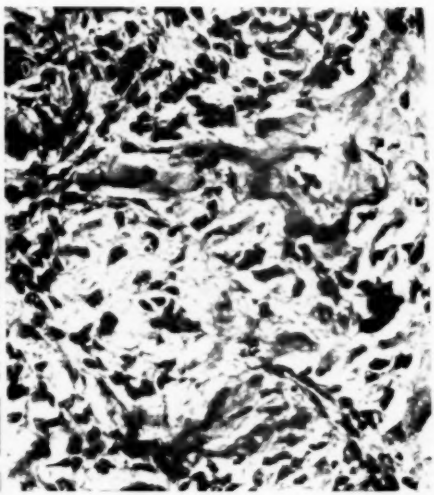

Fig. 4. Photomicrograph of poorly differentiated osteoblastic osteosarcoma from humerus of 22-year-old woman. Patient alive 10 years after amputation. Hematoxylin-eosin. × 240.

3 000 r). The first of these cases does not belong to the series and is only mentioned for the purpose of illustration. In the specimens of both these cases a great diminution of the vascularity of the tumor as compared to those that had not been irradiated was noted. The tumor that received the highest dose presented histologic evidence of necrosis of the vessel walls with extravasation of blood. The second sarcoma, of the chondroblastic type, had intact vessels with only focal necrosis of the walls (Fig. 2). The decreased vascularity of the sarcomas after irradiation is probably due to necrosis and regressive changes in the tumor, perhaps supplemented by increased bone production or calcification in the malignant tissue.

It is well known that the histologic pattern of osteosarcoma varies greatly. We have classed our cases, as DAHLIN, into osteoblastic, fibroblastic and chondroblastic types depending on the dominant tissue element present. In the cases subjected only to biopsy, or in which only one or two tissue sections were available, the classification was often difficult and arbitrary; two such cases were unclassifiable. Commonly two of the elements were present, and in 17 specimens each of the three types was represented. This classification was selected as a means of determining the influence on prognosis of the histologic type. Differences in prognosis had been observed by DAHLIN.

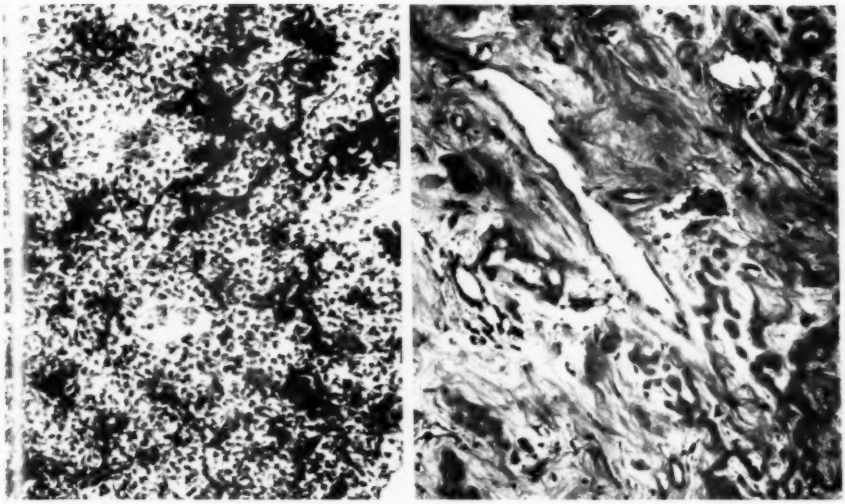

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Fig. 5. a) Biopsy specimen of osteoblastic osteosarcoma from distal femoral metaphysis of 16-year-old boy. Hematoxylin-eosin. × 100. b) Amputation after irradiation. Extensive irradiation effect with a few large, bizarre, nuclei in the hyalinized stroma; osteoid pattern still present. Patient alive 7 years later. Hematoxylin-eosin. × 100.

Of the 94 classifiable tumors, 66 (68.7 %) were of the osteoblastic type, 15 (15.6 %) of the chondroblastic and 13 (13.8 %) of the fibroblastic type. A grading of the osteosarcomas into degrees of malignancy was not attempted. An examination of the microscopic preparations left the impression that degree of differentiation bore little relationship to the survival of the patient; histologically the neoplasms of the 5-year survivors were indistinguishable from those in which the patient died in a short time (Figs 3, 4 and 5). PRICE (1952) reported a distinct correlation between the grade of malignancy of the osteogenic sarcoma and survival. Most of the 5-year survivors were among those with tumors he classed as grade I but the work was based on 36 cases that included periosteal sarcomas and fibrosarcomas.

*Clinical features.* The situation of the tumors in the series was as follows: in the femur in 39 cases, tibia in 26, humerus in 13, pelvis in 5, ulna in 3, fibula in 3, bones of the feet in 2, ribs in 2, and in the sternum, clavicle and scapula in 3 cases. Fifty-five (57 %) of the lesions were located in the region of the knee. The age distribution is in accordance with other published series. Fifty-seven (60 %) of the patients were male and 39 (40 %) were female.

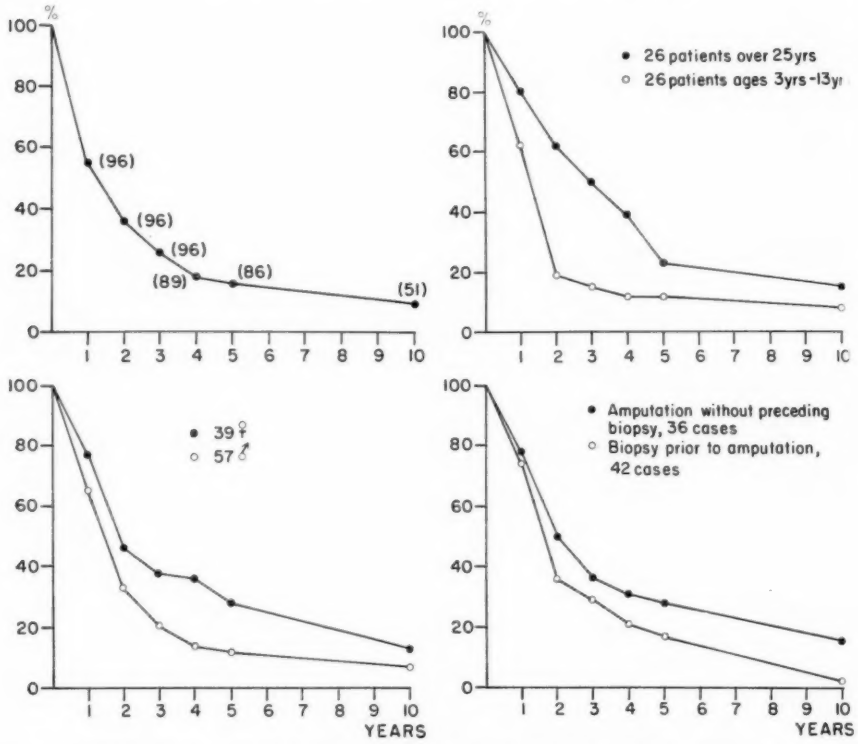


Fig. 6. Survival curves. a) Total material. Figures within parentheses indicate number of cases at follow-up. b) Survival related to age, c) related to sex, and d) related to biopsy.

Calculation of the survival rates has been made using both the time of histologic diagnosis and the onset of symptoms as the starting point (Fig. 6). In a few cases the histologic diagnosis was not established for some time after the symptoms began; often these patients were treated by irradiation therapy first and later by amputation. The differences of the survival rates when computed from the start of symptoms or from the time of histologic diagnosis were, however, insignificant. Of the 86 cases eligible for 5-year survival 16 (18.5 %) lived more than 5 years from the time of diagnosis. Of the 51 cases eligible for 10-year survival 9 (17.6 %) lived more than 10 years. The 5-year survival covering all 96 cases was 16.6 % and will probably increase as there are three cases alive for more than 3 years and these have a fair chance of surviving. The 5-year survival is thus not much different from that reported by DAHLIN. Enquiries regarding 18 patients who lived more than 5 years

ter the start of symptoms showed that 10 were still living. Eight had died; of these died of the tumor and 3 of other causes. In one case the cause of ath was uncertain. Nine patients have survived more than 10 years from the rt of symptoms. Six of these are alive; 2 died from causes other than the rcoma (autopsy), and in one the cause of death was unknown.

We do not believe our material is large enough for a careful statistical alysis of subgroups of cases, but we have divided the material into different ctors that may be related to survival. The age of the patients possibly had me effect on survival. Twenty-six of the cases were more than 25 years old, d 26 cases were in the age group of 3 to 13 years. The age group 14 to 24 ars was excluded; the two groups containing the oldest and the youngest atients were used for comparison. The older group had a somewhat better rvival rate than the younger (Fig. 6). HAYLES et coll. (1960) reported a 2.2 % five-year survival for children with osteosarcoma, the figures being sentially the same as those for the adults. In our material the survival rate as slightly better for women than for men (Fig. 6).

Preoperative irradiation had an unexpected effect in this material. The rvival rates of the preoperatively irradiated 50 cases and the 28 directly perated cases were identical at one, two and three years but were better for he directly operated cases at four, five and ten years. We cannot fully explain his finding. A few patients in the preoperatively treated group refused amputa- ion and were then irradiated but had an amputation later when the tumor progressed further. In these cases the delay in amputation increased the risk f spread of the tumor. Possibly this was true also in those cases in which mputation was not delayed more than was necessary for the irradiation.

Thirty-six of the cases had amputation or resection done without prior biopsy. There was a possible difference in the survival of the patients when compared to those who had had a preliminary biopsy (in most cases incisional) (Fig. 6).

The chondroblastic and fibroblastic tumors possibly had a slightly better survival rate than the osteoblastic ones, which is in accordance with DAHLIN's findings. Females predominated in the group of patients with fibroblastic osteosarcoma (10 of 13), while there were more males among those with chondroblastic osteosarcoma (10 of 15), and in the osteoblastic osteosarcoma group (43 of 66).

No difference in survival could be detected between those patients having sarcomas situated distal to the knee and elbow joints as compared to those in which the tumor was located in the humeri or femori. This is in contrast to the good survival rate observed by DAHLIN (1957) and HAYLES (1960) in osteosarcomas of the tibia. There was no obvious difference between the survival of patients with small or large tumors. The size of each tumor was measured in the earliest roentgenograms and ranged from 3 cm to 23 cm with 8.8 cm as the average diameter.

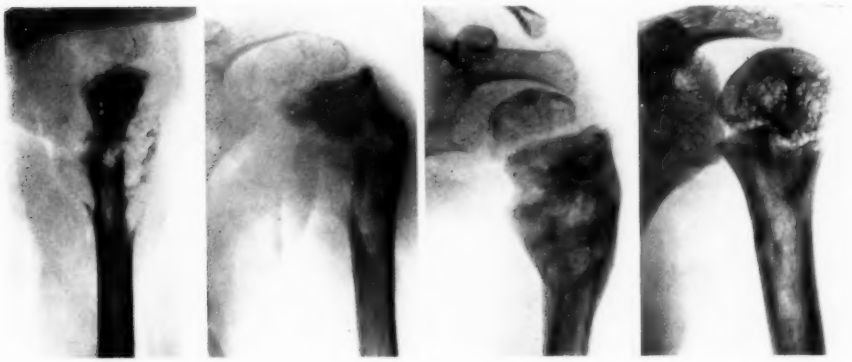

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Fig. 7. Chondroblastic osteosarcoma in boy aged 3 years and 9 months. Irradiation following biopsy. Patient alive after more than 10 years. a) and b) Humerus before treatment a. p. and lateral. c) Five months after irradiation. Extensive calcification in tumor area. d) Five years after treatment. Slight deformity of the bone remains but no evidence of tumor.

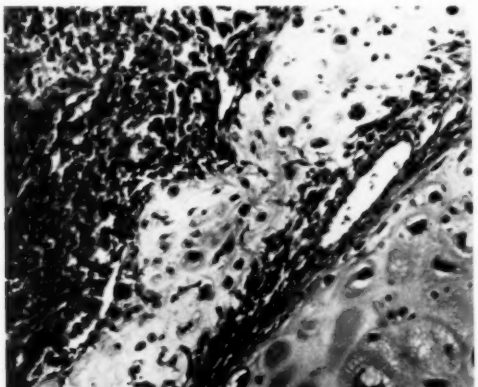

Fig. 8. Same case as in fig. 7. Biopsy specimen. Poorly differentiated chondroblastic osteosarcoma. Hematoxylin-eosin. × 100.

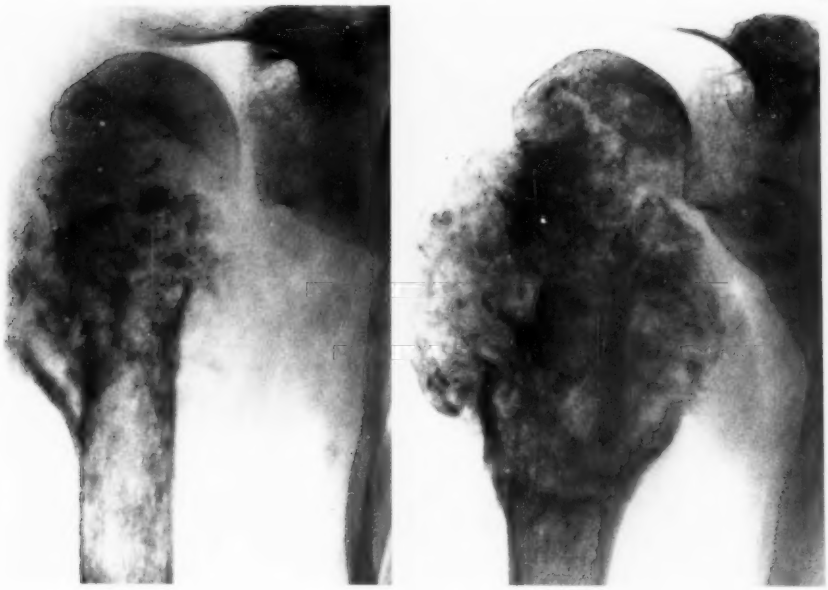

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Fig. 9. Chondroblastic osteosarcoma in a 10-year-old boy with extensive calcification after irradiation. a) Before, b) 3 months after treatment.

## Treatment

No standard method of therapy was used for our cases; treatment had greatly varied but was predominantly surgical. Seventy-eight patients had either amputation or resection of the affected part; 18 had biopsy only and were treated with irradiation. Among these 18 patients were a number in which the location of the tumor made a surgical procedure difficult or impossible. Patients treated only with irradiation therapy had a poorer survival rate than those treated surgically. It should be noted, however, that the irradiation doses in these 18 cases were not high; the tumor dose rarely exceeded 3 000 r and was thus not of the order of 8 000 r recommended by CADE.

The effect of irradiation on the tumor was observed roentgenologically in several of the cases. Calcification and ossification in the tumor increased in many and in several could best be detected in the soft tissue component of the tumor (Fig. 7); in a few instances the tumor became surrounded by a shell of calcium (Fig. 9).

One of the cases treated by irradiation is unique. The patient was a boy

Fig. 10. Osteoblastic osteosarcoma in 14-year-old girl. Typical roentgenographic appearance with sclerosis, diffuse streaks of tumor bone in the extra-osseous parts of tumor, bone destruction, and periosteal reaction. Patient died of pulmonary metastases 3 months after onset of symptoms.

Fig. 11. Osteoblastic osteosarcoma from head of fibula of 31-year-old man. Well-defined destruction and expansion of bone. Atypical appearance. The patient lived 15 years after local resection and died from bronchial carcinoma (autopsy).

aged 3 years and 9 months with a chondroblastic osteosarcoma of the upper end of the humerus. After incisional biopsy, doses of 1 000 r to an anterior field and 1 000 r to a posterior field were given. A great deal of calcification appeared in the tumor area. The bone remodelled well and the patient became symptom-free (Figs 7 and 8). The patient is alive without symptoms 12 years later and only slight deformity of the bone remains. It is noteworthy that this patient was the youngest in our group and that the post-irradiation sclerosis of the tumor was the most marked of the series.

Two patients who had only local resection of the affected part survived more than ten years. The tumor in one was located at the lower end of an ulna and in the other at the upper end of a fibula (Figs 11 and 19).

A comparison was made between five patients under 10 years of age and an equal number over 30 years of age who had received roentgen therapy and had follow-up roentgenograms. Reviewing the pre- and post-irradiation films of the two groups, one gained the impression that calcification was more marked in the younger age group.

## Roentgenographic findings

The initial roentgen films were available for study in all except 2 cases. The following features were seen in the roentgenograms: bone destruction in 72

Fig. 12

Fig. 13 a

Fig. 13 b

Fig. 12. Osteoblastic osteosarcoma from femoral diaphysis of 44-year-old woman. Well-delineated area of destruction with only slight periosteal reaction. Atypical appearances. Patient died from mitral stenosis after amputation. No tumor found at autopsy.

Fig. 13. a) A. p. and b) lateral. Another tumor, histologically and radiographically similar to that in fig. 12, from tibia of 18-year-old man who died 6 months after amputation.

cases, bone sclerosis within the bone in 57, radiating or diffuse bone formation in the soft tissue component in 57, destruction of the cortex in 56, and periosteal bone formation in 59 cases.

In 24 cases, bone destruction, sclerosis and new bone formation in the soft tissue component and periosteal bone formation were all present (Fig. 10). These cases could be diagnosed as osteosarcoma on the basis of the routine roentgen examination.

The films were studied to determine how many of the cases could be diagnosed roentgenographically as osteosarcoma. Ninety-two cases were used for this study and it was felt that 59 (64 %) could be classed as osteosarcomas. As a generalization, it can be said that two-thirds of all osteosarcomas may be diagnosed with reasonable certainty from the roentgenograms alone. Sixteen of the remaining cases presented ill-defined areas of destruction of the bone. Two of these had appearances suggestive of Ewing's sarcoma and

Fig. 14. Osteosarcoma of benign appearance. Well-defined area of destruction in calcaneus of 27-year-old man. Amputation performed 2 years after curettage. Specimen from latter considered benign. Patient died 5 years after amputation from pulmonary metastases.

Fig. 15. Osteosarcoma simulating a fracture through a bone cyst in 6-year-old boy. Slight sclerosis in the metaphysis might suggest the true diagnosis.

in one case the pelvic bone appeared to be the site of a metastasis. Thirteen of the 16 cases merely presented roentgen features of a malignant tumor. The roentgenographic appearances of 6 of the 92 cases were those of a benign tumor. In 2 cases, they were typical of a giant cell tumor (Fig. 11), and in 2 smooth-walled cystic areas were evident in the femoral and tibial diaphysis (Figs 12 and 13); a tumor of the calcaneus was represented by a well-defined area of destruction (Fig. 14). The appearance in one case with a fracture of the upper end of the humerus was very much like a solitary bone cyst (Fig. 15) and only after careful retrospective examination of the metaphysis and its comparison with the epiphysis could sclerosis be detected. Two cases of acetabular osteosarcomas were difficult to detect because of the small area of involvement, in fact in one case the tumor had been overlooked in the original films. In the remaining 9 of the 92 cases the roentgenographic appearances varied widely and had no distinctive features; 4 of the cases were considered to be definitely malignant and in 4 others the possibility of a benign tumor was considered. The remaining case, an osteosarcoma of a cervical rib, had no obvious roentgenographic changes. In a few cases, the course and appearances suggested that the osteosarcoma had arisen in a pre-existing lesion of unknown character (Fig. 16).

Fig. 16. Fibroblastic osteosarcoma following slowly progressing lesion for 9 years in tibia of 31-year-old man. Patient died of pulmonary metastases 4 months after amputation.

Fig. 17. Osteosarcoma in femur of 30-year-old woman. Atypical tumor which is partly osteolytic and partly bone producing. Patient alive 16 years later.

Of the 16 cases surviving five years, 11 had typical changes (Figs 17, 18 and 19). No differences were found between these 11 cases and the other typical osteosarcomas. Five of the cases had appearances that were atypical. Three of these were considered as malignant tumors and two as possibly benign. As a group however, the 16 did not differ much from those which

Fig. 18. Osteoblastic osteosarcoma distally in femur of 9-year-old girl. Irradiation, and 5 months later amputation, as tumor had progressed. It had just penetrated cortex. Patient alive 11 years later.

Fig. 19. Osteoblastic osteosarcoma in 44-year-old woman. Tumor predominantly bone producing. Patient alive 19 years after resection of ulna.

did not survive 5 years. The initial roentgen examination gives no indication as to the ultimate fate of the patient.

*Unusual cases.* One of our cases was similar to sclerosing osteogenic sarcomatosis reported by Mosely and Bass (1956). The tumor was first detected in the proximal metaphysis of the left humerus of a 12-year-old girl. Marked new bone formation was present with similar changes in the same bone on the other side (Figs 20 and 21). The patient lived for 9 months after the tumor was diagnosed and died with lung and kidney metastases and involvement of several bones.

A second case of multiple bone involvement by osteosarcoma also occurred in a 12-year-old girl. Two lesions, one in a femur and one in a humerus were examined by biopsy and diagnosed as fibroblastic osteosarcoma. This patient lived two and a half years and died of lung metastases (Figs 22 and 23).

A woman, aged 41, had for ten years had a slowly growing tumor with gradually increasing pain and swelling in the region of the proximal part of the

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Fig. 20. Multiple foci of osteoblastic osteosarcoma in 12-year-old girl. Autopsy 9 months after onset of symptoms showed wide-spread metastases in skeleton and lungs. a) Bone producing primary tumor fills the greater part of the medullary cavity and surrounds the shaft of the left humerus. b) Several discrete lesions in right humerus.

tibia. The limb was amputated and the growth proved to be an osteogenic sarcoma. The patient died two years later. The roentgenographic appearances in this case were almost identical with those in another case with the same

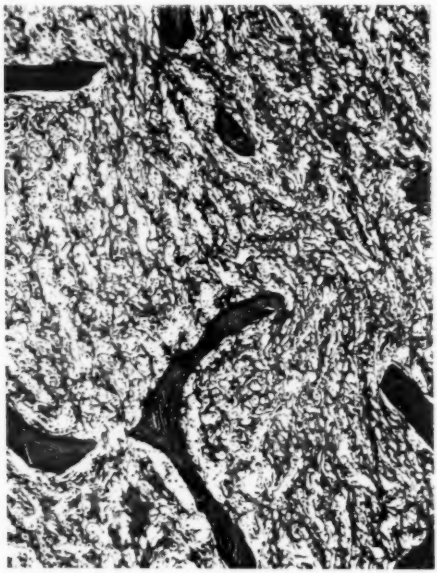

Fig. 21. Same case as in fig. 20. Representative section of the tumors, an osteoblastic osteosarcoma. Hematoxylin-eosin × 40.

localization in our bone tumor registry. The latter case was, however, classified as a fibrosarcoma. Both these cases were similar to the case published by HALL, BERSACK and VITOLO. The long history suggests that it is possible that the tumor in these cases was secondary to an antecedent benign lesion of the tibia (Fig. 16).

## Conclusions

The prognosis in osteosarcoma has long been regarded as very poor. When COVENTRY and DAHLIN published a five-year survival rate of 19.3 %, this figure was generally considered as surprisingly high. Our figure of 18.5 %, however, supports these authors. Although our material was not large enough for a careful statistical analysis, we have noted some trends. The prognosis seems to be slightly better for women than for men and also slightly better in the older age group (above 25 years) than in the children. Fibroblastic tumors were more common in women than in men; possibly this explains in part the better survival for women.

Preoperative irradiation slightly decreased the survival rate. Mention should be made that preoperative irradiation as given to the patients in this study varied greatly in quality and quantity. There was no planned program such

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Fig. 22. Fibroblastic osteosarcoma with multiple skeletal foci in 12-year-old girl. Patient died 2.5 years after onset of symptoms. a) Osteolytic process in femur suggesting a giant cell tumor. b) Lesion in a humerus.

as carried out by CADE. For these reasons our observations do not necessarily reflect the benefits or lack of value of irradiation therapy in osteosarcoma. The youngest of our patients is alive more than 10 years after irradiation therapy only.

Even though our figures indicate a possible increased risk of biopsy, we feel that the basic principle of preliminary biopsy of a tumor prior to surgical or irradiation therapy should be maintained. Until a definitive diagnosis can be made without a biopsy, or until proof of an added danger of biopsy is forthcoming, the risk of the procedure should be accepted. In the case of a tumor of bone biopsy carefully performed by a skilled operator carries a minimum risk of spread of cancer cells.

Approximately two-thirds of all osteosarcomas present typical roentgenographic appearances. A few may appear benign and some may simulate other malignant tumors, e. g. Ewing's sarcoma and giant cell tumor of bone.

## SUMMARY

Ninety-six cases of osteosarcoma are reviewed. The majority of the tumors were situated in the region of the knee. The five-year survival rate was 18.5 %. Patients aged 25 or over

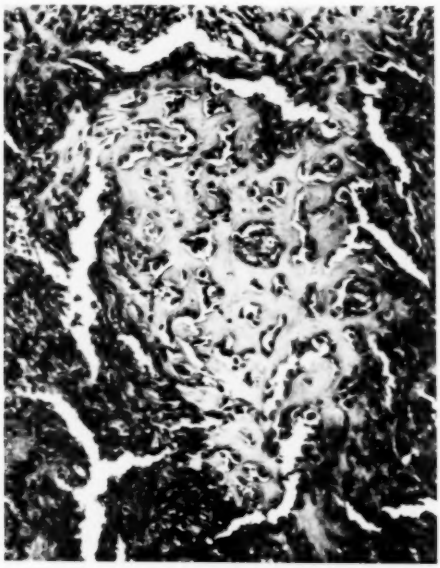

Fig. 23. Same case as in fig. 22. Tumor predominantly fibroblastic with foci of malignant osteoid. Hematoxylin-eosin. × 100.

had a better survival rate than the younger age groups. The roentgen appearances of osteosarcomas are not always characteristic; a few of the growths may appear to be benign and some may simulate other malignant tumors of bone. The treatment is discussed.

## ZUSAMMENFASSUNG

Sechsundneunzig Fälle von Osteosarkom werden gesichtet. Die Mehrzahl der Tumore war in der Kniegegend lokalisiert. Die fünf Jahre-Überlebensrate war 18.5 %. Patienten über 25 Jahre haben eine bessere Überlebensrate als die jüngeren Altersgruppen. Das Röntgenbild von Osteosarkomen ist nicht immer charakteristisch; einige scheinen benign zu sein, andere können anderen malignen Knochentumoren ähnlich sein. Die Therapie wird besprochen.

## RÉSUMÉ

Les auteurs présentent quatre-vingt-seize cas d'ostéosarcome. La majorité de ces tumeurs siégeaient dans la région du genou. Le taux de survie à cinq ans était de 18.5 %. Les malades âgés de 25 ans ou plus ont eu un taux de survie supérieur à celui des groupes d'âges plus jeunes. L'aspect radiologique des ostéosarcomes n'est pas toujours caractéristique; un petit nombre de ces tumeurs ont un aspect bénin et d'autres peuvent simuler d'autres tumeurs malignes osseuses. Les auteurs étudient le traitement.

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# TOMOGRAPHY OF THE AUDITORY OSSICLES

by

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The rise in the number of road accidents, with frequent traumatic injury to the skull, has considerably increased the incidence of fractures of the temporal bone. The bone is usually fractured longitudinally, as Fischer et coll. found in 80 per cent of all their cases. As mentioned e. g. by Kettel (1957) in his description of an operation for the decompression of the facial nerve in cases of post-traumatic facial palsy, it is particularly this kind of fracture of the petrous part of the temporal bone which results in dislocation of the auditory ossicles. It follows that cranial trauma is frequently followed by deafness or impaired hearing. Grove (1939) found ear symptoms with impairment of hearing in 32.2 per cent of 150 cases of cranial fractures.

Another factor contributing to impaired hearing in the past was the inadequate surgical technique employed, which sometimes led to dislocation of the incus.

The introduction of the operation microscope into otology radically altered the chances for performing tympanoplastic operations. The absence of diagnostic aid sometimes forces the otologist to perform explorative tympanotomy as the conventional roentgen examination of the temporal bone, even with the Schüller, Stenver, Runström, Towne, and Chaussé projections, usually pro-

Aided by a grant from the King Christian X Foundation. Submitted for publication 22 November 1960.

Fig. 1. Tympanic cavity seen from within with the ossicles in the normal anatomical position.

vides information only of cholesteatoma, pneumatisation and inflammatory changes, but rarely reveal pathologic changes in or near the ossicles.

The development of tomography has fundamentally altered the basis of radiologic diagnosis in otology. By the use of suitable apparatus the tympanic cavity, atticus and antrum, the internal and external auditory meatus, the cochlea and semi-circular canals, the course of the facial canal, and the ossicles may all be defined. Pre-operative tomography of the temporal bone has thus enabled otologists to plan a tympanoplastic operation and avoid exploration. Tomography of the ossicles is of particular practical significance in congenital deformities, cholesteatoma, osteitic lesions and dislocations, as a tympanoplastic operation often makes it possible to establish an osseous chain of sound transmission.

*Anatomy.* A comprehensive knowledge of the anatomic relationships is a prerequisite for evaluating tomograms of the ossicles. Fig. 1 represents a bone dissection in which the topography of the ossicles in shown. The handle and head of the malleus are located slightly in front of and more laterally than the long process and body of the incus. Sound is transmitted via the malleus and incus to the stapes, the base of which rests on the oval window. The handle of the malleus is connected with the tympanic membrane, and the neck of the malleus is held in position by both the anterior and posterior ligaments; the stapes is kept in place in the oval window by its annular ligament. The incus, however, is retained by only a few strands of ligament, including some from the body of the incus to the fossa for the incus, just as the incudostapedial joint is but loosely bound. This is the weak point in the chain of the ossicles and dislocations of this joint as a result of trauma consequently lead to a displacement between the incus and the stapes.

Fig. 2. Frontal tomography. Normal cut anteriorly through the tympanic cavity (3), external auditory meatus (1), spur (2), cochlea (4), pharyngo-tympanic tube (5), internal auditory meatus (6), jugular fossa (7), facial canal (10) and malleus (m).

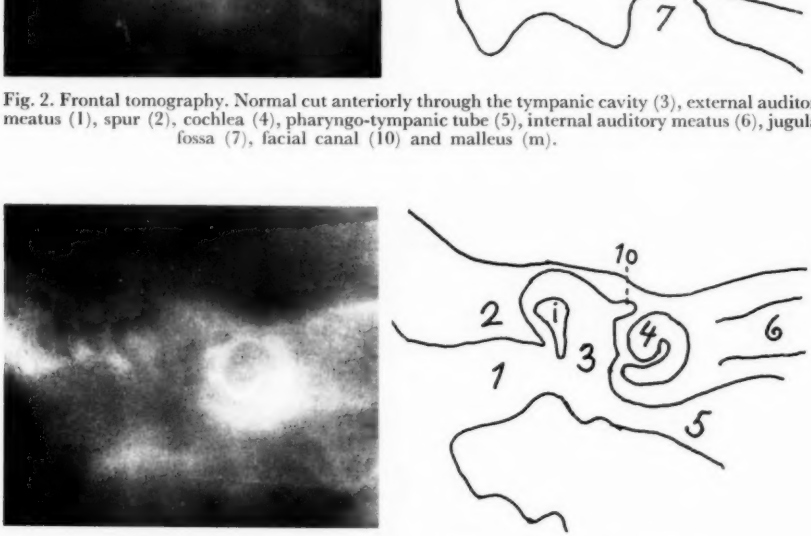


Fig. 3. As in fig. 2 but cut placed 2 to 3 mm more posteriorly. Incus (i).

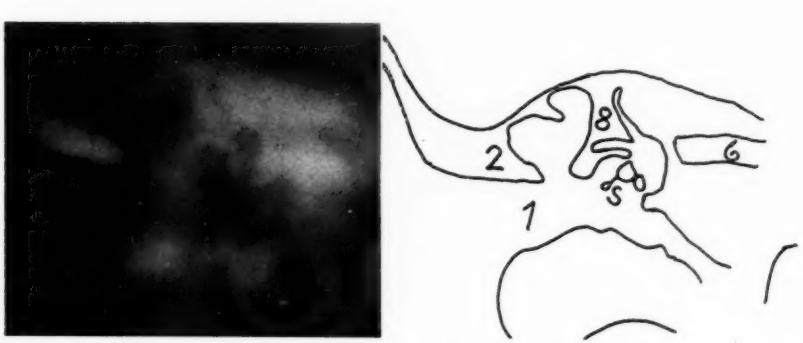


Fig. 4. As in figs 2 and 3 but cut placed through semi-circular canal (8), stapes (s).

## Technique

Massiot's polytome has been found particularly suitable as a tomograph as hypocycloid movement produces very good blurring of structures lying outside the plane desired; furthermore, the polytome can produce quite thin cuts, the thickness of the cut with the hypocycloid movement being 1.3 mm. In describing the curve, the tube traverses a distance of 4.5 m in 6 seconds and the greatest angle of deflection is 48°.

For demonstration of the ossicles we find it most suitable to perform tomography of the temporal bone with frontal and lateral sections.

For frontal tomography, the patient is placed in the supine position so that the median plane of the head and a line joining the external canthus of the eye and the tragus lie at right angles to the plane of the table. The central beam is directed towards the glabella, as the two sides are projected on the same film to facilitate comparison. The anterior part of the tympanic cavity is visible on a cut at the level of the root of the tragus (Fig. 2). Laterally, the external auditory meatus (1) is seen to be bounded below by the tympanic part of the temporal bone, and above by the so-called spur (2) with the tympanic groove. Medially the external auditory meatus is limited by the tympanic membrane which continues into the handle of the malleus. The head of the malleus (m) juts like a club into the middle of the attic, the intermediate ossicle, the incus (i), being shown in cuts from one to a few millimetres posterior to this (Fig. 3). The cochlea (4) appears in both this and the preceding tomogram and the vestibule and semicircular canals (8) are outlined in cuts immediately behind the cochlea (Fig. 4). The stapes (s) lies in the same plane but is often difficult to demonstrate.

For lateral tomography, the patient is placed in the prone position with the head rotated so that a line through both external auditory canals lies at right angles to the table. To make it easier for the patient to maintain this position, the shoulder on the side away from the table is lifted and the arm flexed while the other arm is extended parallel to the body. Contrary to LANGFELT (1960), we prefer to let the ear under examination be the one further from the table, as the focusing is considerably easier, the position less uncomfortable for the patient, and the distances from the focus to plane of cut and from the plane of cut to film remain constant. The central beam is directed towards the root of the tragus and, by placing the plane of the cut between 2 and 2.5 cm medial to the lateral face of the mastoid process, tomograms are obtained through the tympanic cavity. Such a section is shown in Fig. 5 in which the cut actually passes through the malleus (m) and the incus (i). It is seen that the body of the incus and the head of the malleus are situated at the same level, and further that the handle of the malleus and the long process of the incus are parallel to each other and lie in the tympanic cavity (3). Behind and above, a small part

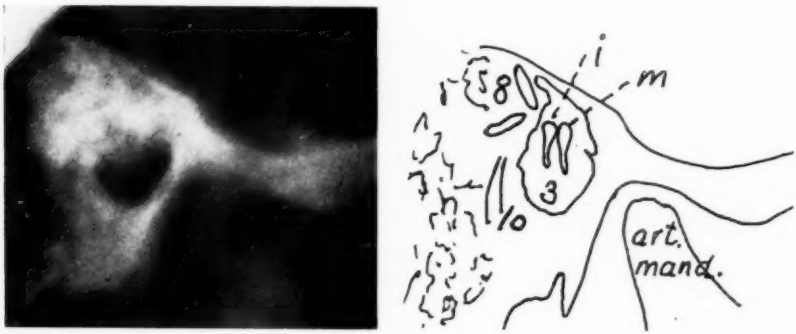


Fig. 5. Lateral tomography. Normal cut through tympanic cavity (3), semi-circular canal (8) facial canal (10), incus (i) and malleus (m). The long process of the incus and the handle of th malleus are parallel.

Fig. 6. Case 1. Frontal tomography showing displacement of incus (i) in relation to malleus (m).

of the semi-circular canals (8) is evident and, behind and below, a small portion of the canal for the facial nerve appears (10). The mandibular joint can be seen lying anteriorly.

As impaired hearing usually causes the patient great distress and often results in mental disturbances and difficulties in social adjustment, it is a matter of great importance to be able to diagnose the cause of the condition. In the following cases of lesions of the ossicles, pre-operative tomography made it possible to determine the changes, and thus plan the required operation for improvement of hearing.

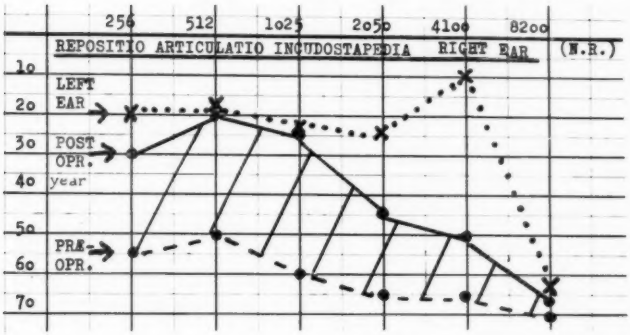


Fig. 7. Case 1. Audiogram showing hearing in left ear . . . (healthy side), and hearing before - - - and after operation ——— in right ear.

## Case reports

*Case 1.* Boy, age 9 years. Six years previously he had sustained a fracture through the right temporal bone (demonstrated in roentgenograms). No note of haemorrhage or discharge. Hearing in the right ear had since been impaired. Otologic examination disclosed that the patient could not hear a whisper at more than 0.10 m (normal 6 m) with the right ear. Hearing on the left side normal. The tympanic membrane of the right ear was intact but with residual scarring. The ossicles were not seen clearly.

Frontal tomography was performed. Cuts through the anterior part of the tympanic cavity (Fig. 6) revealed the presence of an ossicle-like structure, presumably the incus (i), lying laterally to the malleus (m). A dislocation must therefore have been present. This was verified at operation, as the chain of ossicles was broken, with the long process of the incus displaced considerably backwards and rotated medially. The incus was successfully reset and articulation with the stapes re-established.

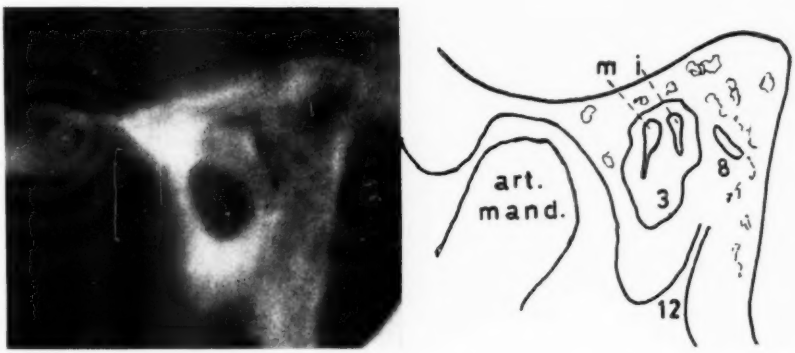


Fig. 8. Case 2. Lateral tomography showing displacement between incus (i) and malleus (m) and divergence between the long process of the incus and the handle of the malleus.

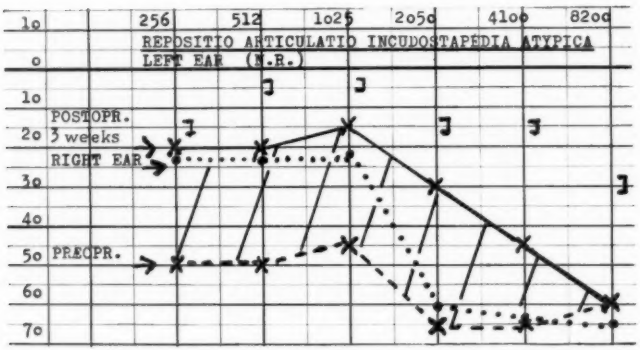


Fig. 9. Case 2. Audiogram showing hearing in right ear before - - - and after operation ———.

The postoperative course was uneventful and the patient could hear a whisper with the right ear at a distance of 2 to 3 m. Audiograms before and after operation are shown in Fig. 7. The improvement in hearing is evident from the curves.

*Case 2.* Boy, age 13 years. When six months old he had undergone bilateral simple mastoidectomy. Hearing had been considerably impaired since the operation and during the previous three or four years, in spite of a hearing-aid, he had had difficulty in keeping up with his class at school. Otologic examination established that a whisper could not be heard at more than 0.2 m; there was scarring of both ear drums. Left lateral tomography (Fig. 8) showed that the body of the incus (i) was displaced downwards so that there was undoubtedly dislocation of the incudo-stapedial joint. It was not possible with certainty to differentiate the incus from the malleus, however, on the right side.

On the strength of the roentgen findings, operation was performed on the left side, and the body of the incus was found to be considerably displaced, with the long process of the incus

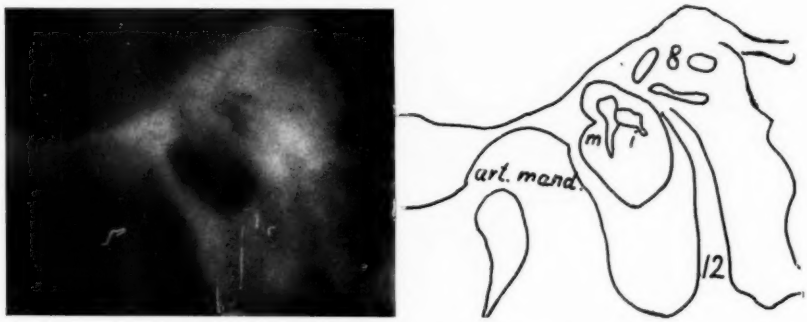


Fig. 10. Case 3. Left lateral tomography showing displacement between incus (i) and malleus (m) and divergence between the long process of the incus and the handle of the malleus.

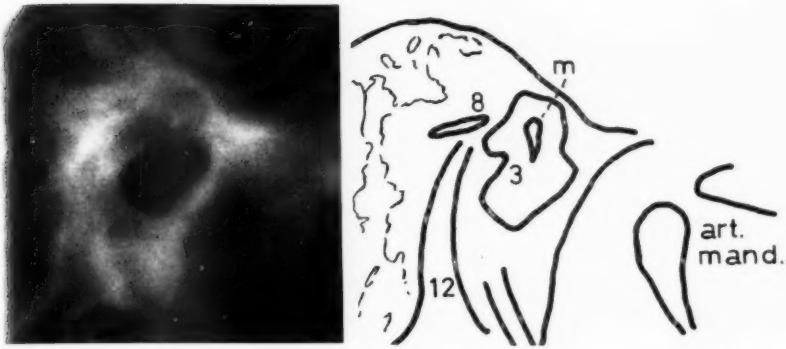


Fig. 11. Case 3. Right lateral tomography. Only the malleus (m) is seen with certainty.

pointing forwards. Reposition of the incus was performed. Recovery was uneventful, and nearly normal hearing was obtained in the operated ear. Audiograms before and after operation are shown in Fig. 9, indicating clearly the improvement in hearing. It is planned to re-examine the patient's right ear at a later date with a view to possible operation on that side.

*Case 3.* Seaman, age 23 years. When two years old he had undergone resection of both mastoid processes; recent impaired hearing had forced the patient to give up his occupation. On physical examination the patient could not hear a whisper at a distance of more than 0.1 m; the ear drums were dull in colour but intact. Left lateral tomography (Fig. 10) showed a downward displacement of the body of the incus (i). On the right side the incus was not visible with certainty, whereas the malleus was clearly seen (Fig. 11). At the subsequent operations it was found that the incus on the left side was dislocated, with the long process directed inwards and forwards instead of downwards and inwards. Reposition of the incus was per-

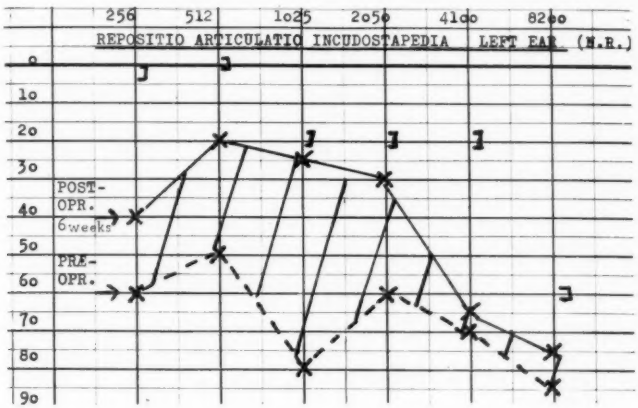


Fig. 12. Case 3. Audiogram showing hearing of the left side before - - - and after operation ———.

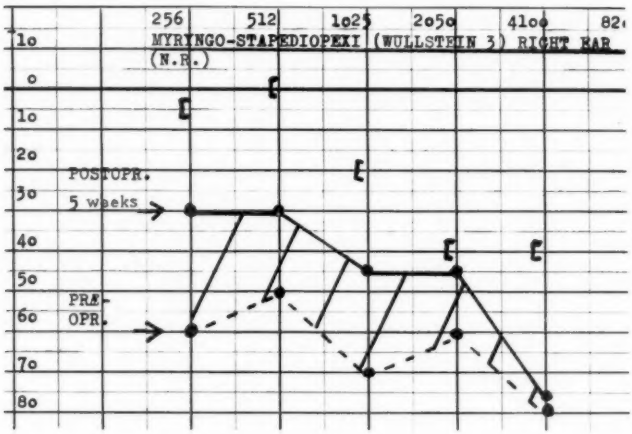


Fig. 13. Case 3. Audiogram showing hearing on right side before - - - and after operation ———.

formed, whereby normal articulation was re-established. Hearing was considerably improved, as the patient was able to hear a whisper at a distance of 4.5 m on the left side, as compared with 0.1 m previously, as mentioned. This improvement is apparent from the audiogram (Fig. 12).

Operation on the right side was also performed, but conditions here were less favourable, as the roentgen examination had already suggested. The incus was found to be totally dislocated, forced downwards, and wedged backwards. It was thus necessary to displace the tympanic membrane onto the stapes. But this also improved the hearing, as the distance at which a whisper was heard increased from 0.1 m to 1.3 m. The audiogram (Fig. 13) shows the improvement in hearing on the right side.

## Discussion

The three case histories show that re-establishment of the chain of ossicles led to considerable improvement in hearing, both in the patient with impaired hearing following trauma and in the patients with impaired hearing caused by earlier inadequate surgical technique, in spite of a lapse of 6, 12 and 21 years, respectively, between the primary trauma and the plastic operation. Operation is facilitated by the fact that reposition need only consist in replacing the articular surfaces of the incudo-stapedial joint, without consideration for scar formation or ankylosis, as the joint does not lose its capacity to transmit sound even though a considerable period of time has elapsed after dislocation. Individual cases of successful operations for dislocation of the incus have been reported in recent years (Bauer 1958, Gisselsson 1958 and Flisberg & Floberg 1960). All these authors performed the operations on an indication of suspected

icle dislocation without being able to verify the diagnosis pre-operatively, as ther by ordinary otoscopy nor by use of Siegle's speculum were they able confirm the condition.

## Conclusion

Tomography of the temporal bone should be carried out at the earliest portunity in cases of impaired hearing following head injuries in order to minate possible dislocation of the ossicles.

## SUMMARY

Tomography enables dislocation of the auditory ossicles following a head injury or ear operation to be demonstrated. The anatomy of the region is described and three typical cases are sented.

## ZUSAMMENFASSUNG

Mit der Tomographie kann eine Dislokation der Gehörknöchelchen durch ein Schädeltrauma oder eine Ohrenoperation demonstriert werden. Die Anatomie wird beschrieben und 3 typische Fälle werden vorgelegt.

## RÉSUMÉ

La tomographie permet de mettre en évidence la luxation des osselets de l'oreille après un traumatisme cranien ou une opération sur l'oreille. Les auteurs décrivent l'anatomie de cette région et présentent trois cas typiques.

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FROM THE CENTRAL ROENTGENDIAGNOSTIC DEPARTMENT (DIRECTOR: PROF. KNUT LINDBLOM), KAROLINSKA SJUKHUSET, STOCKHOLM, SWEDEN.

# VERTICAL CASSETTE STAND

by

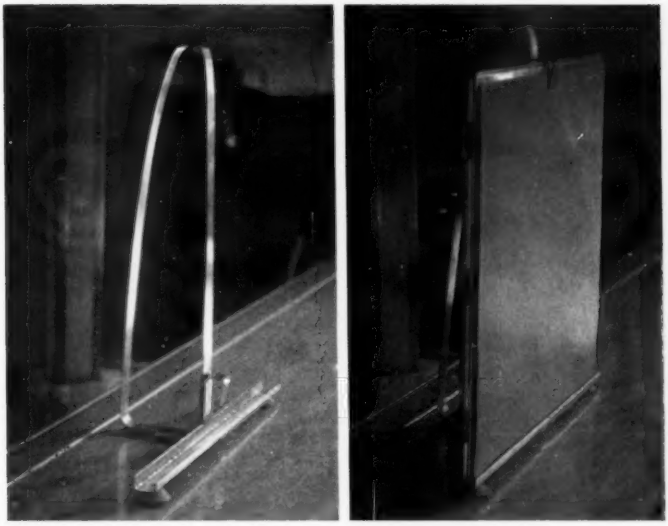
PEKKA SOILA

The horizontally directed roentgen beam fulfills an important role in many roentgenologic examinations. So may for example a search for fluid levels in the paranasal sinuses, the chest and the abdominal cavity be considerably aided by this technique. Vertical cassette supports of different constructions which in some way can be attached to the side of the examination table are commercially available; they are often provided with a fluorescent screen. However, cassettes are frequently supported only by sandbags and this often results in unstable and more or less oblique positioning of the cassette with consequent inaccuracy in the examination.

A free-standing vertical cassette stand of stainless steel is shown in Fig. 1. This stand is easily manipulated and the cassette can rapidly be placed in the required position, as close as possible to the part to be examined. It can be built for any standard cassette size. A schematic drawing is shown in Fig. 2, giving the measurements for cassette sizes of 24 × 30 cm and 35 × 35 cm.

The problem of maintaining the balance of the cassette stand during an examination has been solved by means of attaching three rubber suction cups to its base. They adhere to the surface of the examination table. Any need for an extension of the stand towards the patient's side is hereby eliminated and the opposite side can be made short enough to leave sufficient room on an examination table for the different positions of the patient.

Submitted for publication 14 December 1960.



a b

Fig. 1. a) Vertical cassette stand. b) Cassette and grid mounted on the stand.

The groove of the cassette stand has been made sufficiently wide to accommodate a grid in front of the cassette, a combination necessary in most examinations. The clip which holds the upper end of the cassette in position is movable along the vertical limb of the stand to facilitate the use of cassettes of various sizes; it also retains the grid. We have found it advisable in practice, however, to have two sizes of supports available, one for the cassette size 24 × 30 cm and another for the size 35 × 35 cm. Provided that the suction cups function well, the only difference between the two stands is the height of the vertical limb, which is indicated by the letters (a) and (b) in Fig. 2.

In case fluoroscopy is required, it may be carried out with the help of a portable screen, whereafter the stand with the cassette can be placed in the desired position.

**Acknowledgement**

This work was carried out during the author's tenure of a James Picker Foundation Research Fellowship at Karolinska Sjukhuset.

## SUMMARY

A vertical casette stand of simple design is described.

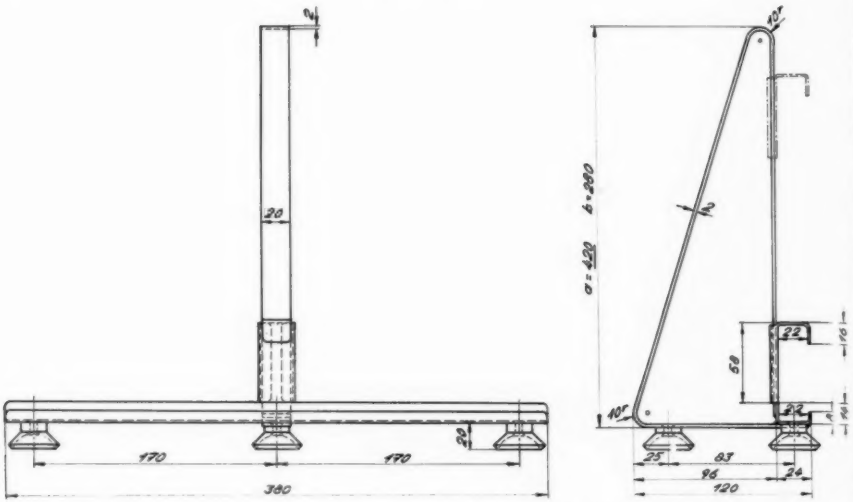



Fig. 2. Schematic drawings showing the constructional details of the vertical stand for cassette sizes 24 × 30 cm and 35 × 35 cm. The figures indicate the measures in millimeters.

## ZUSAMMENFASSUNG

Es wird ein einfacher vertikaler Kassettenhalter beschrieben.

## RÉSUMÉ

Description d'un support de cassette vertical de construction simple.

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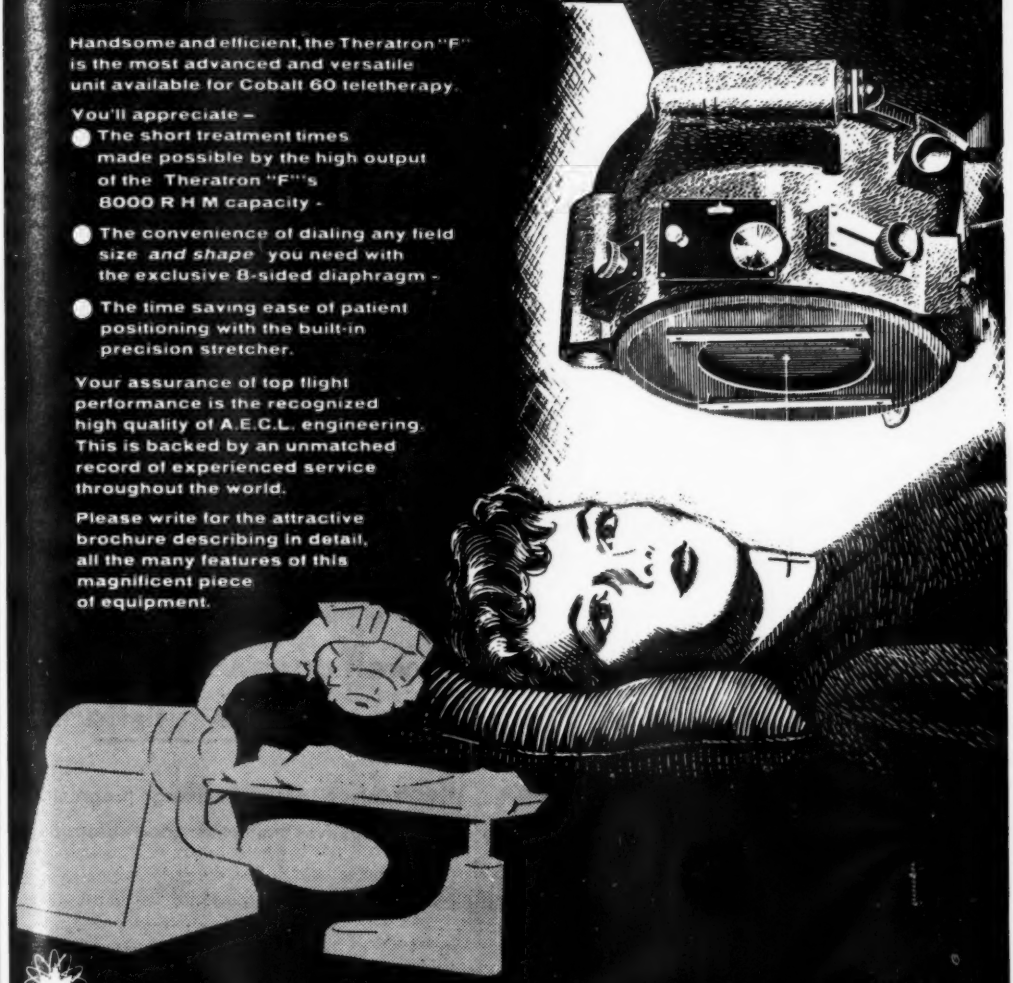

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# PALLIATIVE TREATMENT OF CARCINOMATOUS EFFUSIONS IN THE PLEURAL AND PERITONEAL CAVITIES WITH RADIOACTIVE GOLD

by

EILER LAMBRETHSEN and ARNE SELL

Cancerous conditions of the pleural and peritoneal cavities are often associated with considerable fluid formation. Previously, the only palliative treatment was repeated tapping, possibly supplemented in the case of susceptible tumours with roentgen irradiation and hormone therapy, although roentgen therapy to large abdominal or thoracic fields often gives rise to severe side effects which prevent the completion of a planned course of treatment.

The introduction of treatment with radioactive isotopes (MÜLLER 1945, 1950) marked a great advance as the method soon proved capable of affecting the fluid formation favourably without any marked side effects. The substance preferred during the past 10 years has been radioactive gold, $Au^{198}$. Let it be emphasized at once that this form of therapy is also merely palliative.

## Physical and Technical data and Material

*Physical data.* $Au^{198}$ emits particle irradiation in the form of electrons as well as electromagnetic irradiation. The mean range of the $\beta$ irradiation in organic tissue is about 0.4 mm. The $\gamma$ irradiation is more penetrating but makes up only a small proportion (about 15 %) of the total energy emitted by the disintegrating gold (BEIERWALTES 1957, LOW-BEER 1950). Thus, the main effect of the $Au^{198}$ administered is obtained through the $\beta$ radiation, and only the superficial layers of the tumour-studded serous membranes are affected. The half-life is 2.7 days.

Work carried out under a grant from the Danish Anti-Cancer League. Submitted for publication 16 September 1960.

*The mechanism of the effect* of the radioactive gold has been more or less established. It consists partly in an action upon the free cancer cells in the effusion (ANDREWS et coll. 1953), and partly in a lethal effect upon the superficial neoplastic lesions on the pleura or peritoneum. Furthermore, it is considered likely that radiation effects upon the serosal blood vessels and lymphatics may contribute to reduced fluid formation. The main portion of the instilled radioactive gold is phagocytized by macrophages and deposited in the serous membranes where it exerts its effect. A part of the gold particles is absorbed via the lymphatics and deposited mainly in the liver and bone marrow. Swedish animal experiments (FRIES et coll. 1959) indicate that the permeability of the serosa to $Au^{198}$ may be altered by the administration of a high-molecular polyphenol (polyphloretin phosphate), so that a smaller quantity of gold is absorbed and reaches the reticuloendothelial system. In addition, the presence of neoplastic tissue on the serosa per se has proved to inhibit the permeability. Administration of such permeability-inhibiting agents might be considered to be indicated mainly in prophylactic treatment with radioactive gold.

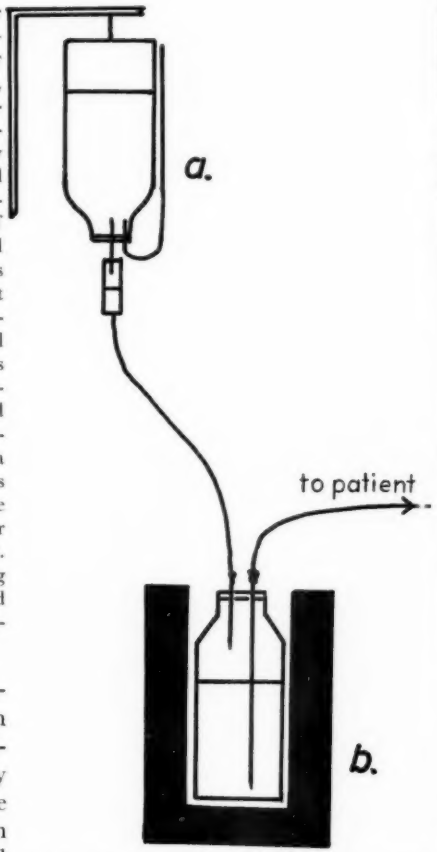


Fig. 1. Instillation set for $Au^{198}$ therapy: Bottle with normal saline furnished with a disposable infusion set (a). Container of lead with vial containing colloidal suspension of $Au^{198}$ (b).

*Technique.* We used a colloidal solution of $Au^{198}$, instilled, after evacuation of the effusion, into the pleural or peritoneal cavity with a suitable quantity (200 to 300 ml) of normal saline. The pleural puncture is performed with an ordinary coarse needle, left in situ until the $Au^{198}$ instillation which follows immediately. We use a trocar for evacuating the ascitic fluid; a few centimetres away from the trocar, an ordinary coarse needle is introduced through the abdominal wall into the peritoneal cavity and through this needle the solution of gold is instilled after removal of the trocar; the wound is closed with agraffes or sutures. This procedure was used in order not to risk leakage and contamination by the radioactive agent. The usual therapeutic dose of $Au^{198}$ is 135 to 150 mC into the peritoneal and 75 to 100 mC into the pleural cavity.

*The installation apparatus* (Fig. 1) was simplified as far as possible in order to obtain the shortest possible instillation period (radiation risk) and the minimun

**Table 1**

*Distribution by site of primary tumour, sex and age*

| Primary tumour | Number of cases | | | Mean age (years) |
|---|---|---|---|---|
| | Females | Males | Total | |
| Breast cancer | 23 | 0 | 23 | 58 |
| Ovarian cancer | 21 | 0 | 21 | 51.6 |
| Cancer of the lung | 0 | 3 | 3 | 64 |
| Cancer of the stomach | 1 | 1 | 2 | 43 |
| Cancer of the pleura | 1 | 1 | 2 | 60 |
| Cancer of the rectum | 1 | 0 | 1 | (66) |
| Sarcoma | 0 | 1 | 1 | (42) |
| Hodgkin's disease | 1 | 0 | 1 | (48) |
| Total | 48 | 6 | 54 | |

risk of contamination, as well as from a consideration of ease in training the personnel. By the technique used, any manipulation of the vial holding the radioactive solution is avoided since it remains in the lead container in which it has been received. After removal of the cover of the lead container, two needles, a short afferent and a long efferent needle, are inserted through the rubber cap of the vial. The short needle is connected, via a disposable infusion set, with a bottle of sterile physiologic saline. The pressure of the fluid in the saline bottle forces the gold solution through the long needle which is connected by rubber tubing with the needle inserted into the pleural or peritoneal cavity. Immediately before the instillation we make sure that the needle is still in the serous cavity by ascertaining that there is a free flow of saline. After the instillation the tubing and needles are discarded but are first kept in a safety compartment in a special container until the activity has subsided (5 or 6 half-lives). Immediately after the instillation the patient is started on a fixed programme of frequent changes in posture in order to secure an even distribution of the radiogold. We have not had an opportunity of 'scanning' the patients with a view to checking the distribution of the isotope but in a few cases we have controlled the distribution by roentgen films.

*Present material.* The series comprises all patients treated with radioactive gold during the period July 1957 to November 1959, a total of 54 (Table 1). The majority were women, the two groups of breast cancer and ovarian cancer being numerically the largest. The average age may also be seen from Table 1. Out of the 54 cases 31 had pleural effusion, 23 peritoneal carcinosis, with effusion in 19, whereas the remaining 4 cases were treated prophylactically because of cancerous seedlings on the serosal surfaces. A total of 84 instillations were administered.

The series is heterogeneous and comprises cases of incurable cancer in a varying general condition.

In analysing the material, we included all the treated patients, also those

**Table 2**

*Effect of $Au^{198}$ therapy (intraperitoneal instillation) assessed on the basis of the frequency of taps*

| Primary tumour | No. of cases | Effect ++ | Effect + | Effect 0 | Survival time in months < 1 | > 1 | > 3 | > 6 | > 1 |
|---|---|---|---|---|---|---|---|---|---|
| Ovarian cancer | 12 | 7 | 1 | 4 | 4 | 8 | 6 | 3 | 1 |
| Breast cancer | 5 | 3 | 0 | 2 | 1 | 4 | 3 | 2 | 1 |
| Cancer of the stomach | 2 | 0 | 0 | 2 | 1 | 1 | 0 | 0 | 0 |
| Total | 19 | 10 | 1 | 8 | 6 | 13 | 9 | 5 | 2 |

who died within a month of the treatment and in whom it was difficult to assess the effect.

## Results

The object of the treatment was to reduce or possibly abolish the frequent considerable fluid formation associated with diffuse carcinosis of the pleural and peritoneal cavity; the patients would be spared repeated taps, and their general condition would be temporarily improved to an extent which would permit the institution of other palliative measures.

It is difficult to assess the results and not easy to set up an objective measure of the effect. We selected the frequency of taps before and after the instillation of $Au^{198}$ as the most important criterion. Like previous authors (SEAL et coll. 1958) we have divided our series into 3 groups: good, moderate, and no effect.

*I. Good effect*, ++: No tap during a period of at least 2 months. This group, however, also includes patients who were tapped within a month of the $Au^{198}$ instillation, if this last tap was followed by a period of at least 2 months without any tapping.

*II. Moderate effect*, +: No tap during a period of a month or two after the instillation.

*III. No effect, 0:* Tapping needed within one month of the instillation. This group also includes the patients who died within a month of the treatment.

For the time being, we shall leave out of the intraperitoneal group four cases which had prophylactic instillations, since they cannot be assessed on the basis of the frequency of taps before and after instillation. This leaves 19 patients (Table 2) treated by repeated taps before the instillation of $Au^{198}$. A good effect was obtained in 10, or 53 %; ignoring 6 desolate cases dying within one month, there was a good palliative effect in 75 %. Table 2 also includes the survival time after the first $Au^{198}$ treatment; about 50 % of the patients survived for more than 3 months and about 25 % for more than 6 months. Only 2 were alive one year after the first instillation.

Four patients were treated with radiogold as a prophylactic measure against the development of ascites. These were cases of ovarian carcinoma in which

**Table 3**

*Effect of $Au^{198}$ therapy (intrapleural instillation) assessed on the basis of the frequency of taps*

| Primary tumour | No. of cases | Effect ++ | Effect + | Effect 0 | Survival time in months < 1 | > 1 | > 3 | > 6 | > 12 |
|---|---|---|---|---|---|---|---|---|---|
| Breast cancer | 18 | 9 | 1 | 8 | 5 | 13 | 10 | 8 | 5 |
| Ovarian cancer | 5 | 4 | 1 | 0 | 0 | 5 | 4 | 2 | 1 |
| Cancer of the lung | 3 | 1 | 0 | 2 | 2 | 1 | 1 | 0 | 0 |
| Cancer of the pleura | 2 | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 0 |
| Cancer of the rectum | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| Hodgkin's disease | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Sarcoma | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| Total | 31 | 16 | 3 | 12 | 7 | 24 | 17 | 12 | 6 |

operation had shown slight peritoneal seedlings of the cancer but no ascites. Two of these patients have later died, 72 and 80 days respectively after treatment and two are still alive without signs of ascites.

Thirty-one cases of carcinomatous pleural effusions were assessed on the basis of the same criteria. The results (Table 3) are in keeping with those of the intraperitoneal treatment, a little over half the cases (51.6 %) responding by a good palliative effect. In this group there were 7 desolate cases, the patients dying within one month of the treatment; if these 7 cases are excluded, a good palliative effect was obtained for about 75 %. From Table 3 it will be noted also that 6 out of the 31 treated patients were alive one year after the first $Au^{198}$ instillation, i. e. about 20 %.

As regards the average survival time after the first $Au^{198}$ instillation, this was 3 months 28 days in the intrapleural group of cases. Nine patients are still alive, 6 of them more than a year after the treatment. Among the intraperitoneal cases the average survival time is 4 months 8 days. Only two patients are alive more than one year after the first treatment. The 4 patients having prophylactic treatment are not included.

On the basis of the intervals between the taps and the quantity of tapped fluid, we tried to calculate the daily fluid formation before and after the $Au^{198}$ instillation in order to obtain yet another objective measure of the effect in addition to the frequency of taps (Fig. 2). This could be done in all cases in the peritoneal group. An unmistakable fall in daily fluid formation was observed in all those showing a good and moderately good effect of the treatment (assessed on the basis of the frequency of taps). In addition, a fall in the daily fluid formation was found in 5 of the patients in whom the intervals between the taps were not lengthened. In other words, an effect of the gold therapy was found in 24 out of 34 intraperitoneal instillations, i. e. about 70 %. In the case of the pleural effusions, similar curves could not be traced in the same proportion of cases, since the evacuation of the effusions could not always be complete.

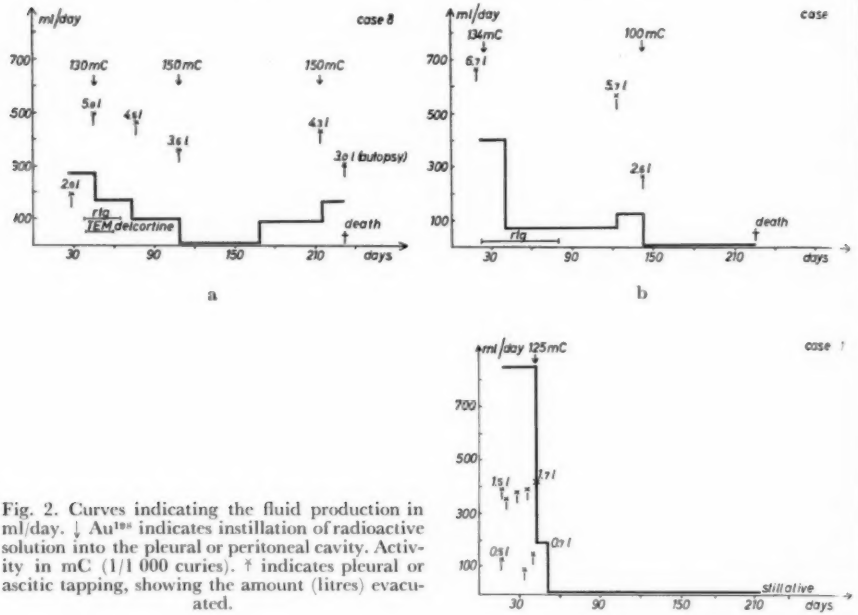


Fig. 2. Curves indicating the fluid production in ml/day. ↓ $Au^{198}$ indicates instillation of radioactive solution into the pleural or peritoneal cavity. Activity in mC (1/1 000 curies). ↑ indicates pleural or ascitic tapping, showing the amount (litres) evacuated.

Even so, we tried to evaluate the 18 instillations after which no effect was recorded or after which the patients died in a month; in 4 of these cases the daily fluid formation decreased.

*Effect of repeated instillations.* The 50 cases of effusion received a total of 80 instillations. A good effect of the first instillation was observed in 60 %. Nineteen patients had two instillations, with a good effect of the second instillation in about 50 %. Eight had 3 to 4 instillations, but only one of them showed a good effect from these last instillations. The intervals between the instillations varied considerably, from 2 or 3 weeks up to several months. The duration of the effect of an $Au^{198}$ instillation cannot be assessed accurately, but it may be estimated as being 2 to 4 months. In 3 or 4 cases we found that after the first, less effective treatment, a long-lasting decrease in fluid formation was obtained after the second instillation (Fig. 2a), when performed 4 to 6 weeks after the first. This indicates that the results may be improved by repeating the $Au^{198}$ application (possibly several times) at relatively brief intervals (2 or 3 weeks) (Hansen & Haug 1959, 1960).

A factor which must be considered in assessing the effect is concurrent treatment (roentgen irradiation, cytotoxics combined with adrenocortical hormone and, in cases of metastatic breast cancer, treatment with sex hormones or adreno-

c ical hormone). Concurrently with a total of 48 instillations leading to a good r onse, 21 cases were receiving other forms of treatment; in other words, in a ost half the cases, supplementary treatment must be expected to have con- t uted more or less to the favourable result, although the curves show that t effect was due mainly to $Au^{198}$. Diminution of major neoplastic lesions was n observed in any case after $Au^{198}$, except in cases receiving supplementary r ntgen therapy. Reviewing the series in order to discover a possible haemo- st ic action of $Au^{198}$, reflected by decreasing blood admixture to the effusion, w found no such effect.

## Complications and Side effects

The complications and side effects were moderate and short-lasting. We g uped the side effects according to the time of their occurrence after the $Au^{198}$ a plication. No complications occurred at the time of the instillation; there w s no record of accidental instillation into the abdominal or chest wall, per- fo ation of the bowel, or pulmonary injury.

During the first days after the instillation, side effects were observed in about h lf the intraperitoneal cases in the form of one or more of the following symp- to ns: pain or heaviness in the abdomen, nausea, vomiting, diarrhoea, and el vation of temperature. In the intrapleural cases the side effects were of a so newhat different nature: increased dyspnoea, cyanosis, cough, nausea, vomit- ing, and elevation of temperature. Well over half the patients complained of on e or more of the named symptoms. These side effects were not particularly se vere and lasted only for a day or two.

A group of secondary complications, or sequelae appearing days or weeks after the instillations, consisted partly of local changes and partly of more generalized effects including haematologic changes and exacerbation of the general condition, in some cases fatal.

A few authors (Dennis et coll. 1956 and Elkins et coll. 1956) have reported ileus as a complication to the intraperitoneal application of $Au^{198}$. We observed this complication in four cases which are reported upon below.

*Case 1.* Female, aged 40, with inoperable ovarian carcinoma and marked ascites. In the course of a 6-month period she received three $Au^{198}$ instillations (130, 150, and 150 mC). Two weeks after the last instillation, she developed obstruction high up in the small intestine from which she died within two weeks. Autopsy showed all the loops of the small intestine to be matted together by adhesions. The peritoneal lining of the intestine was greatly thickened and of a dull pale blue colour. There were also adhesions between the liver, spleen, and diaphragm. No visible tumour tissue. Histologic examination revealed thickening of the peritoneum and depositions of gold particles as well as diffuse carcinosis.

*Comment.* The patient died of adhesion ileus. Autopsy did not reveal major tumour masses which might have induced this condition. Since the patient had not been subjected to surgery, it must be considered likely that the adhesions were induced by $Au^{198}$; these developed in the course of a period which could not have been longer than 5 months. The third and last $Au^{198}$ instillation possibly provoked the ileus.

*Case 2.* Female, aged 50, with ovarian carcinoma and peritoneal dissemination. Postoperative roentgen therapy to the abdomen followed later by triethylene melamine (TEM), and a little more than a year later $Au^{198}$ (138 mC) intraperitoneally because of ascites, and at the same time further roentgen therapy and TEM combined with prednisone. A good effect upon the ascites was observed, but at the end of 2 months it increased again and another instillation of $Au^{198}$ (135 mC) was given. Twenty-four hours later the patient developed ileus and died; immediately prior to the last instillation, she had been feeling relatively well and had no abdominal symptoms. Autopsy was not performed.

*Comment.* Unmistakable exacerbation occurred after $Au^{198}$, with ileus provoked presumably by the instillation.

*Case 3.* Male, aged 55, with a history of gastric resection for ulcer in 1950. In January 1959 ileus preceded by several attacks of sub-ileus. Exploratory laparotomy revealed peritoneal carcinosis and ascites as well as numerous adhesions. Immediately after the operation, $Au^{198}$ (100 mC) was administered intraperitoneally and 12 days later 150 mC. One week after the last instillation, the patient died apparently from an obstruction in the small intestine. Autopsy showed the transverse colon, stomach, spleen, pancreas, liver, and the anastomosed loops of the small intestine to be embedded in a great mass of tumour tissue and adhesions; in addition there was diffuse peritoneal carcinosis.

*Comment.* The widespread adhesions were presumably due to the first operation. The tumour tissue in the abdomen may explain the intestinal obstruction although the instillation of $Au^{198}$ may have provoked it.

*Case 4.* Female, aged 47, with ovarian carcinoma and peritoneal carcinosis. After a palliative operation, she had roentgen irradiation to the abdomen, TEM, and $Au^{198}$ (175 mC) intraperitoneally with a good effect upon the ascites. The $Au^{198}$ instillation was repeated 2.5 months later, 150 mC being followed by repeated roentgen therapy and TEM. Twenty days after the last instillation, ileus developed (confirmed by roentgen examination) to which the patient succumbed within 10 days. Autopsy showed dissemination of cancer on the parietal peritoneum, a large neoplastic infiltration in the true pelvis, and adhesions of the small intestine. The bowel was of a dull pale blue colour, and there was unmistakable thickening of the subserous tissue. There was no single obstruction, but numerous stenosed areas were present in the small intestine due to the adhesions.

*Comment.* The patient died of an adhesion ileus. The adhesions may have been due to the original operation, but they may also have been caused by the $Au^{198}$. The last instillation of $Au^{198}$ may have provoked the ileus.

All these four patients had received at least two instillations of $Au^{198}$. A varying length of time after the latter instillation they developed ileus to which they succumbed. The first two patients had no major tumour masses in the abdomen, whereas the latter two had neoplastic masses which may have caused the ileus. $Au^{198}$ may perhaps give rise to adhesions or may provoke ileus in cases with adhesions or major neoplastic masses; the spontaneous course of widespread abdominal carcinoma, with a marked tendency to ileus, must be borne in mind.

Among other local secondary complications, we observed two cases of long-lasting diarrhoea, as reported upon below.

*Case 5.* A youngish woman who had been subjected to operation for ovarian carcinoma. The operation revealed minute carcinomatous seedlings on a limited area of the peritoneum lining the true pelvis. No ascites. Three weeks later, the patient had a prophylactic instillation of $Au^{198}$ (150 mC) into the peritoneal cavity. No complications occurred in connection with

instillation, but one week later she began to have frequent, sanguineous, slimy, watery tions accompanied by rectal tenesmus. Rectoscopy revealed haemorrhagic proctitis and a ium enema a spastic, dehaustrated colon. The patient was successfully treated with predni- one and hydrocortisone enemas.

*Comment.* It seems reasonable to suppose that the $Au^{198}$ was responsible for the syndrome gesting ulcerative colitis. Adhesions caused by the gynaecologic laparotomy may possibly ve given rise to the formation of pockets around the descending colon, so that the radio- ive solution had no chance of becoming evenly distributed.

*Case 6.* Female, aged 70, with peritoneal carcinosis and ascites (primary tumour of the breast). eated twice with intraperitoneal $Au^{198}$ with a favourable effect. After a third instillation, out 6 months later, she developed, within two weeks, non-sanguineous diarrhoea which sisted for about a month. Neither rectoscopy nor roentgen examination of the colon was formed. The patient died in a state of cachexia 3 months after the last instillation of $Au^{198}$.

*Comment.* Probable adhesions with pockets around the colon, induced by the previous $Au^{198}$ illations.

Among the intrapleural cases, local secondary complications were extremely re. Pleural empyema occurred in two cases. In one of these, the empyema was immediately associated with the instillation of $Au^{198}$, presumably because of lack of asepsis. In the other case, empyema was present at the time of the $Au^{198}$ application; the condition deteriorated, and the patient died within a month. The instillation of radioactive solution in this case was no doubt contra-indicated.

*Haematologic changes.* Fifteen cases treated with radiogold exhibited decreased white cell and/or platelet counts in the peripheral blood. In ten of them the changes may be ascribed to concurrent treatment (TEM combined with roentgen therapy), and the decrease was not more marked or more prolonged than in other cases treated with TEM and roentgen rays alone (JØRGSHOLM 1959). Only 5 of the cases treated exclusively with $Au^{198}$ showed moderate haematologic changes and these presented no problems.

We wished to ascertain whether the $Au^{198}$ therapy had in some cases aggravated the patients' condition and possibly speeded the downhill course. Out of the intraperitoneal cases, a total of 10 died within one month of the instillation. In 4 of them the treatment appeared to have aggravated the condition and precipitated the fatal outcome. All these cases had large neoplastic masses in the abdomen and all four were in poor general health. Out of the intrapleural cases, 8 died within one month of the treatment. All were in a poor general condition, with severe cyanosis only moderately eased by pleural taps. Several of them required permanent administration of oxygen. In 5 of these debilitated patients, we felt that the treatment had aggravated the condition but that we should not draw too far-reaching conclusions.

In order to find out why in some cases the treatment did not lead to the desired result we analysed the cases that failed to respond, basing our assessment upon the frequency of taps. In the intraperitoneal group, 14 instillations were failures. In 10 of these cases, the general condition was poor at the time

of the instillation, and in addition large neoplastic masses were palpable in th abdomen of 8. All 10 patients died within 39 days after the $Au^{198}$ instillation The remaining 4 were in a fairly good general condition without palpabl tumour masses in the abdomen; they died of other causes within one mont of the treatment (agranulocytosis, pulmonary atelectasis, pulmonary embolu and ileus). It would therefore seem as if a poor general condition and larg neoplastic masses in the abdomen hold out little hope of successful treatmen the expectation of life being short. Even in these cases, however, a few hav responded by some decrease in the daily fluid formation. As far as the intra pleural cases are concerned, 18 instillations failed. Thirteen of these patient were in poor general condition, having severe dyspnoea and requiring in mos cases permanent administration of oxygen. A number of the patients als had widespread neoplastic lesions in the lungs (primary cancer of the lung o metastases) or large cancerous seedlings on the pleura (4 cases). It must be as sumed that a poor general condition and severe dyspnoea refractory to pleura puncture afford little prospects of effective $Au^{198}$ instillation. Furthermor cases with extensive pleural seedlings are not suited for this treatment.

The series includes two cases of pleural mesothelioma. One responded wel to the instillation at the outset but two subsequent instillations were ineffec tive; at that time there was massive coating of the pleura with tumours. In th other case there was no effect upon the frequency of thoracocenteses but a moderate action upon the daily fluid formation. Others (HANSEN et coll. 195 KLINGERMAN et coll. 1955) have reported favourable results of $Au^{198}$ in pleura mesothelioma.

*Protective measures.* The precautions which must be observed in order to re duce the radiation risk to the staff as far as at all possible will be briefly con sidered. This problem arises in the following three situations: in the course of instillation, during the time immediately after the instillation, and in the event of autopsy.

It is important that the period of instillation and the manipulations with the radiogold should be as brief as possible. Furthermore, one must safeguard against leakage from the patient as already mentioned. It is often expedient to place the patient with the punctured side uppermost during the inflow and while the needle is being withdrawn. We did not carry out special measure ments, but by a pocket dosemeter we ascertained that during an instillation procedure of 10 to 15 minutes, the whole-body dose was about 10 to 15 mr.

While the patient is lying in the ward after the instillation, the protection problems concern almost exclusively the nursing staff. It must be enjoined upon the nurses to spend the briefest possible time in the vicinity of the patient and to stay as far from him as possible. In no case was the permitted weekly dose of 300 mr exceeded or even approached. It is advisable to keep the patient in hospital until the activity has decreased to about 30 mC. In several cases the

$Au^{198}$ instillations were given on an out-patient basis (9 intraperitoneal and 6 intrapleural instillations), and in such an event the patient's next-of-kin must of course be informed of the situation and warned of the danger to children, pregnant women, and spouses of the fertile age.

Autopsy on patients who die a short time after the instillation presents a special problem, due in part to the direct radiation risk to the autopsy staff, and in part to the risk of contaminating utensils. Manuals dealing with radiation protection (BRAESTRUP 1958) recommend that autopsy, without special precautions, on bodies containing more than 5 mC radioactive isotope should not be performed; in cases in which the activity is higher, it is necessary to assess the time to be allowed for each postmortem in order not to exceed the permissible radiation dose.

## Discussion and conclusion

Our experience of radioactive gold therapy is in accordance with the reports of others (HANSEN et coll. 1959, 1960, DIAMOND 1958), i. e. a good palliative effect in about 50 % of cases of carcinomatous effusions in the peritoneal and pleural cavity. It is worth stressing that this special procedure has gradually become an important link in palliative treatment, but let us not underestimate concurrent measures which have often contributed to the favourable result. The main indication is rapid re-accumulation of the effusion. The dosage level was 135 to 150 mC $Au^{198}$ into the peritoneal cavity and 75 to 100 mC into the pleural cavity. Several examples showed that repeated instillation a few weeks after the first led to a surprisingly long symptom-free period. Supplementary measures were roentgen irradiation to localized major neoplastic lesions as well as hormone therapy and chemotherapy in suitable cases. It is important to select patients in the best possible general health, since the therapeutic results are considerably less favourable if the patients are in a poor general state. In the latter cases we believe that gold therapy may contribute in aggravating the condition. It is our experience, moreover, that in the presence of large, widespread tumour masses no particular effect can be expected. As far as the intraperitoneal cases are concerned, there is also the risk of complications in the form of adhesions leading to ileus. For this very reason, more reserve is now being displayed in using radioactive gold for intraperitoneal instillation and Thiotepa appears to be preferred as has been emphasized particularly by Swedish authors (KARLSTEDT). In cases of pleural carcinomatosis, the same effect appears to be obtainable by the intrapleural administration of Thiotepa (HOLIN 1960). In patients with severe dyspnoea which persists after evacuation of the effusion, the effect of radiogold is very slight, at times even unfavourable. In toto, it may be stated that a poor general condition and large tumour masses, particularly in the abdomen, afford if not direct contraindications then at least every reason for a conservative approach.

## SUMMARY

A series of 54 cases with carcinomatous effusions were treated by instillations of radioactive gold into the pleural or peritoneal cavity. A good palliative effect was obtained in about 50 %. Side effects and complications are reported and particular attention is drawn to the risk of adhesions leading to ileus. The less favourable effect in cases in which the general condition is poor and in which large neoplastic masses are present in the pleural or peritoneal cavity is stressed.

## ZUSAMMENFASSUNG

Vierundfünfzig Fälle mit karzinomatösen Ergüssen wurden mit Instillation von Radiogold in die Brust- oder Bauchhöhle behandelt. Ein guter palliativer Effekt wurde in etwa 50 % der Fälle erzielt. Nebenwirkungen und Komplikationen werden berichtet und besondere Aufmerksamkeit wird auf das Risiko von Adhäsionen gerichtet, welche zu einem Ileuszustand führen können. Der weniger günstige Effekt in Fällen, die einen schlechten Allgemeinzustand haben und bei denen grosse Tumormassen in der Pleura- oder Peritonealhöhle vorliegen, wird hervorgehoben.

## RÉSUMÉ

Une série de 50 cas d'épanchements cancéreux ont été traités par injections d'or radioactif dans les cavités pleurale ou péritonéale. On a obtenu un bon effet palliatif dans environ 50 % des cas. Les auteurs décrivent les effects secondaires et les complications, et attirent particulièrement l'attention sur le risque d'adhérences provoquant une occlusion. Ils insistent sur l'effet moins favorable dans les cas où l'état général est mauvais et où il existe de grosses masses néoplasiques dans les cavités pleurale ou péritonéale.

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# SIGNIFICANCE OF UPTAKE OF RADIOACTIVE IODINE IN SALIVARY GLAND IN CARCINOMA OF THE THYROID

by

BENGT SKANSE, INGE GYNNING and INGE HEDENSKOG

Radioactive iodine is widely used in the examination of cases of malignant thyroid diseases and is particularly useful for locating functioning metastases. We have recently observed that the uptake of radioactive iodine by a salivary gland may be misleading. A search of the literature failed to disclose any reports of similar observations.

## Case report

A 29-year-old woman, who had had a lump in her neck for 10 years, underwent thyroidectomy with left radical cervical dissection in 1957; the growth proved to be a follicular adenocarcinoma with lymph node metastases. Preoperative studies had revealed that the uptake of radioactive iodine in the region of the tumour was lower than in the apparently uninvolved right lobe of the thyroid. The lymph node metastases in the operation specimen were found to have taken up radioactive iodine, the uptake per gram of wet tissue varying from 0.19 to 0.75 per cent of the dose of $I^{131}$ administered. Four months later a lump was felt in the right lobe of the thyroid and the patient was submitted to right total thyroidectomy with dissection of the cervical lymph nodes. Microscopy revealed no signs of malignancy. A part of the right submandibular salivary gland was removed at the operation as it appeared to be enlarged and was thought to be involved.

A pea-sized nodule appeared on the left side of the neck a year later; it failed to concentrate iodine and was removed. Microscopy proved the nodule to be a granuloma caused by a suture.

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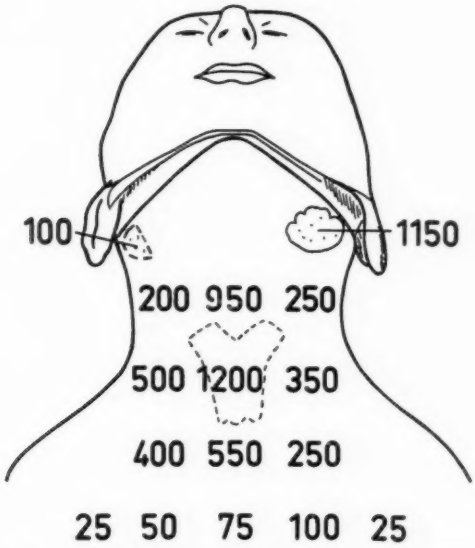


The results of in vivo counting over the neck. The numbers represent the net counts per minute obtained over the areas indicated. Background of 150 counts per minute.

About nine months later a hard lump, the size of a hazelnut and not tender to palpation, was found in the left upper cervical region, and on withdrawal of the replacement therapy with triiodothyronine the patient rapidly became myxedematous. Location studies with $I^{131}$ indicated that the mass concentrated iodine (see figure). The total uptake in the neck was less than 2 per cent of the administered dose. Conventional laboratory equipment was used for the examination: a heavily shielded gamma scintillation detector (P-20C, Tracerlab) with a $1'' \times 1.5''$ D thallium-activated sodium iodide crystal in combination with a clinical scintillation collimator (P-25, Tracerlab) with the small-angle insert (24 mm diameter bore). As there appeared to be evidence of a metastasis from the thyroid, the left submandibular region was explored and the left submandibular salivary gland and surrounding tissues were removed en bloc; microscopy revealed a normal salivary gland apart from some lymphocytic infiltration, lymph nodes with slight inflammatory signs, and a foreign body granuloma. Analyses for radioactivity disclosed a much higher concentration of $I^{131}$ in the salivary gland (0.0064 per cent of administered dose per gram of wet tissue) than in the lymph node (0.0009 %/g tissue) and the foreign body granuloma (0.0007 %/g tissue). Since the bulk of the operation specimen consisted of salivary gland it was obvious that the latter contained practically all of the radioactivity in the region.

## Discussion

The salivary glands in man possess the power to concentrate iodide (SCHIFF et coll. 1947, et alii). The ratio between the concentration of $I^{131}$ in the salivary

glands and that in the serum varied between 4.6 and 11.2 in a case which had been given a large dose of radioactive iodine 59 hours before death (COHEN & MYANT 1959). STEIN et coll. (1957) observed that the salivary $I^{131}$ concentrations were highest in hypothyroid subjects and decreased as the thyroid activity increased. It is therefore hardly surprising that in our case which had been subjected to total thyroidectomy a considerable part of the radioactive iodine in the neck region was found in the submandibular salivary gland. Assuming that the submandibular gland had a normal weight of about 10 g (it was not weighed), it contained about 0.06 per cent of the $I^{131}$ administered. Since the total activity in the whole neck was less than 2 per cent, the iodide concentrating capacity of the salivary gland must have been considerable. The difference in iodine uptake between the left and the right submandibular region was most likely due to the removal of the right submandibular salivary gland at the second operation. The iodide concentrating power of salivary glands may vary widely not only between different species but also in one and the same species (COHEN & MYANT 1959).

A granuloma in the region of a normal submandibular salivary gland was mistaken for a functioning thyroid metastasis. Such possibilities of error should be borne in mind in the search for aberrant thyroid tissue and thyroid metastases with radioactive iodine techniques.

## SUMMARY

A case is reported which draws attention to the salivary glands as a source of error in the location of functioning thyroid metastases in the upper cervical region.

## ZUSAMMENFASSUNG

Die Verfasser berichten einen Fall, welcher die Aufmerksamkeit auf die Speicheldrüsen als eine Fehlerquelle bei der Lokalisation funktionierender Thyreoideametastasen in der oberen Halsregion richtet.

## RÉSUMÉ

Les auteurs présentent un cas qui attire l'attention sur la source d'erreur dans la localisation de métastases thyroidiennes sécrétantes dans la région cervicale supérieure que peuvent constituer les glandes salivaires.

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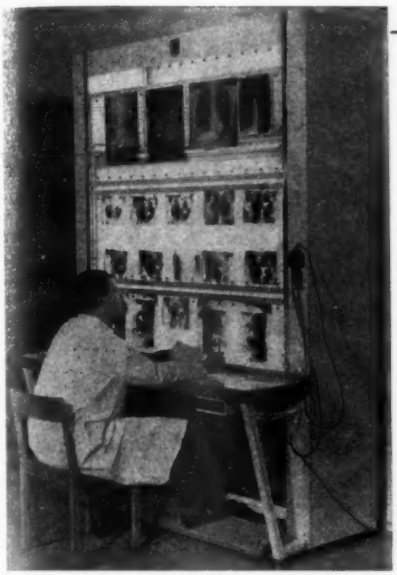

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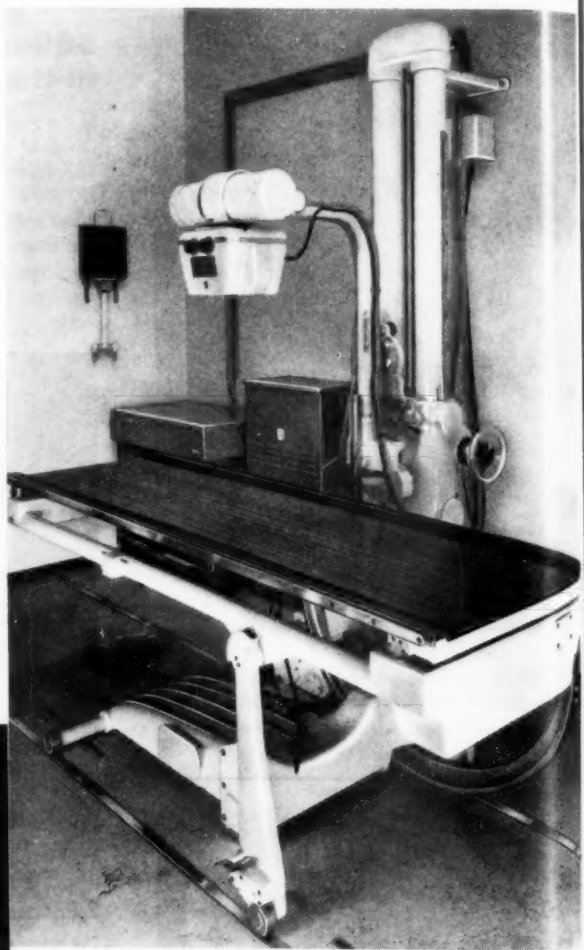

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UNIVERSITY OF CAMBRIDGE, ENGLAND.

# $H^3$ INCORPORATION INTO ASCITES TUMOUR AND TISSUE CULTURE CELLS EXPOSED TO SYNKAVIT AND ITS TRITIATED ANALOGUE

by

IRMELIN SIMON-REUSS

In the clinical and laboratory studies of chemical radiosensitisers, which have been in progress in Cambridge since 1946, about 170 compounds have been examined (MITCHELL 1960a). The best chemical radiosensitiser is still the first compound selected for investigation. This is tetrasodium 2-methyl-1: 4-naphthaquinol diphosphate, our Compound 1 (Synkavit, Roche Products Ltd).

Further, this compound is the carrier molecule for the radioactive drug which is being developed (HORWITZ et coll. 1959, MITCHELL 1960b, MARRIAN et coll. 1961). For this purpose, tetrasodium 2-methyl-1: 4-naphthaquinol (6-T) diphosphate is being used. This tritiated compound of high specific activity was first prepared by the method of reductive dehalogenation of tetra 6-iodo-2-methyl-1: 4-naphthaquinol diphosphate (EVANS & MARRIAN, to be published).

The preparation used in the present investigation contains 13 curies of tritium per millimole; all the tritium is firmly bound in the molecule. It is to be noted that one atom of tritium per molecule corresponds to a specific activity of 29.1 curies per millimole, so that the preparation studied here in the experiments contains 0.446 atoms of tritium per molecule. It may be mentioned that preparations of still higher specific activity containing almost exactly 1 atom of tritium, which is firmly bound, per molecule are now readily

Submitted for publication 6 February 1961.

**Table 1**

*Tissue cultures treated with tritiated compound 1, tritiated water and tritiated water + 'cold' synka it.*

| Nos | Material | Tritium | Activity mC/ml | Concentration of chemical compound | Time of treatment in min | ARG exposure time in hrs | Per cent of cells labelled | Autoradiographic gr in counts per cell | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Weak 10—60 | Medium 80—200 | He vy 22( — 1 ( 0 |
| 620, 583 | E. M. F. | Tritiated compound 1 | 60 | $5 \times 10^{-3}$ M | 2 | 24 | All | | | - |
| 584 | | | | | 5 | 24 | All | | | - |
| 585 | | | | | 10 | 24 | All | | | - |
| 586 | | | | | 20 | 24 | All | | | - |
| 561 | | | 0.12 | $1 \times 10^{-5}$ M | 30 | 72 | All | + | | - |
| 562 | | | | | 60 | 72 | All | + | | - |
| 563 | | | | | 80 | 72 | All | | + | - |
| 564 | | | | | 120 | 72 | All | | + | - |
| 687 | E. M. F. | Tritiated water | 50 | | 2 | 24 | | Not countable, too m ch background | | |
| 689 | | | 0.1 | | 5 | 24 | | Not countable, too m ch background | | |
| 721 A—723 B | | | 0.1 | | 15 | 72 | 13.0 | + | | |
| 725 A—727 B | | | 0.01 | | 30 | 72 | 18.5 | + (mostly in dividing cells) | | |
| 688 | E. M. F. | Tritiated water | 50 | + 'cold' Synkavit $5 \times 10^{-3}$ M | 2 | 24 | | Not countable, too m ch background | | |
| 690 | | | 0.1 | $1 \times 10^{-5}$ M | 5 | 24 | | Not countable, too m ch background | | |
| 622 | H. A. | Tritiated compound 1 | 60 | $5 \times 10^{-3}$ M | 2 | 24 | All | | | - |
| 703—704 | | | 1.2 | $1 \times 10^{-5}$ M | 2 | 24 | All | | + | |
| 625 | | | 0.12 | $1 \times 10^{-5}$ M | 30 | 72 | | Not countable, too m ch background | | |
| 705—706 | H. A. | Tritiated water | 0.1 | | 15 | 24 | None | | | |
| 707—708 | H. A. | Tritiated water | 0.1 | + 'cold' Synkavit $1 \times 10^{-5}$ M | 15 | 24 | None | | | |
| 621 | HeLa | Tritiated compound 1 | 60 | $5 \times 10^{-3}$ M | 2 | 24 | All | | | - |
| 624 | | | 0.12 | $1 \times 10^{-5}$ M | 30 | 72 | All | | | + nd m re |
| 699 | HeLa | Tritiated water | 50 | | 2 | 5 | None | | | |
| 101 | | | 0.3 | | 80 | 72 | 38.5 | | + | |
| 701 | | | 0.1 | | 5 | 5 | None | | | |
| 700 | HeLa | Tritiated water | 50 | + 'cold' Synkavit $5 \times 10^{-3}$ M | 2 | 5 | None | | | |
| 702 | | | 0.1 | $1 \times 10^{-5}$ M | 5 | 5 | None | | | |

**Table 2**

*Ehrlich ascites tumour cells treated in vitro*

| s | Tritium | Activity mC/ml | Concentration of chemical compound | Time of treatment min | ARG exposure time hrs | Per cent of labelled cells | Autoradiographic grain counts per cell | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Weak 10—60 | Medium 80—200 | Heavy 220—1 000 |
| 62 -631 | Tritiated compound 1 | 60 | $5 \times 10^{-3}$ M | 2 | 48 | | Not countable, too much background | | |
| 56 -566 | | 0.12 | $1 \times 10^{-5}$ M | 30 | 72 | All | | + | |
| 56 -570 | | | | | 96 | All | | | + |
| 57 -572 | | | | 60 | 72 | All | | | + |
| 57 -576 | | | | | 96 | | Not countable, too much background | | |
| 577 -578 | | | | 80 | 72 | All | | | + |
| 579 -588 | | | | | 96 | | Not countable, too much background | | |
| 639 -650 | Tritiated water | 50 | | 2 | 24—120 | None | | | |
| 663 -674 | | 1.0 | | 5 | 24—120 | None | | | |
| 645 -662 | Tritiated water | 50 | + 'cold' Synkavit $5 \times 10^{-3}$ M | 2 | 24—120 | None | | | |
| 675 -686 | | 1.0 | $1 \times 10^{-5}$ M | 30 | 24—120 | None | | | |

available from the Radiochemical Centre, Amersham, Buckinghamshire, England (catalogue number, TRA-72). Here, it is relevant to mention that evidence of some degree of selective concentration of this compound in certain human tumours has been obtained in clinical investigations especially after intra-arterial injection.

The present investigation is a quantitative autoradiographic study to determine the time and degree of incorporation and the details of the distribution of the tritium of tritiated Compound 1 in certain adult, embryonic and malignant cells. In these experiments we have used tissue cultures of human amnion, of the HeLa strain of human uterine cervical carcinoma and embryonic mouse fibroblasts, together with the cells of the Ehrlich mouse ascites tumour (hyperdiploid strain of Lettré) both in vitro and in vivo. The control experiments were made with comparable concentrations of tritium in the forms of tritiated water ($T_2O$) diluted in saline and of tritiated water diluted with solutions of non-radioactive Compound 1 ('cold' Synkavit). In considering the details of the experiments, it may be useful to refer to the summary of the results in Tables 1, 2 and 3.

**Table 3**

*Ehrlich ascites mice treated intraperitoneally in vivo*

| Nos | Tritium | Final concentration in ascites fluid | | Time of treatment min | ARG exposure time hrs | Per cent of labelled cells | Autoradiograph grain counts per c ll | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Activity mC/ml | Concentration of chemical compound mg/ml | | | | Weak 10—60 | Medium 80—200 | He vy 22 — 1 00 |
| 633—638 | Tritiated compound 1 | 20 | 0.65 | 5 | 48 | | Not countable, too much background | | |
| 691—698 | | 3 | 0.1 | 5 | 24 | All | + | | |
| 709—711 | | 0.43 | 0.015 | 20 | 6 | All | + | | |
| 712—714 | | | | | 24 | All | + | | |
| 740—743 | | 0.24 | 0.008 | 20 | 24 | All | + | | |
| 744—745 | | | | | 42 | All | + | | |
| 752 | Tritiated water | 1.25 | | 20 | 24 | None | | | |
| 753—754 | | | | | 48 | None | | | |
| 755 | | | | | 72 | None | | | |
| 756 | | | | | 96 | 14.5 | < 5 | | |
| 757 | | | | | 144 | 19.2 | < 5 | | |
| 715 | | 0.022 | | 20 | 5 | None | | | |
| 716—717 | | | | | 24 | None | | | |
| 718 | | | | | 48 | None | | | |
| 719 | | | | | 72 | None | | | |
| 720 | | | | | 144 | None | | | |
| 746 | | 0.022 | | 30 | 24 | None | | | |
| 747—748 | | | | | 48 | None | | | |
| 749—751 | | | | | 72 | 14.0 | < 6 | | |
| 758 | Tritiated water | 1.7 | + 'cold' Synkavit 0.17 | 20 | 24 | None | | | |
| 759—760 | | | | | 48 | None | | | |
| 761 | | | | | 72 | None | | | |
| 762 | | | | | 96 | 7.8 | < 5 | | |
| 763 | | | | | 144 | 8.4 | < 5 | | |

## Experimental methods

*Radioactive preparations.* The specimens of tritiated Compound 1, batch No. 20 (P/N No. 012601), contained 13 curies per millimole. The solution used contained 120 millicuries of tritium and 3.9 mg of the compound per millilitre.

The control experiments were carried out with tritiated water of specific activity 1 curie per ml diluted with either Ringer's solution or/solutions of Compound 1 ('cold' Synkavit in Ringer's solution to give specific activities of the final solutions comparable with those of the tritiated Compound 1.

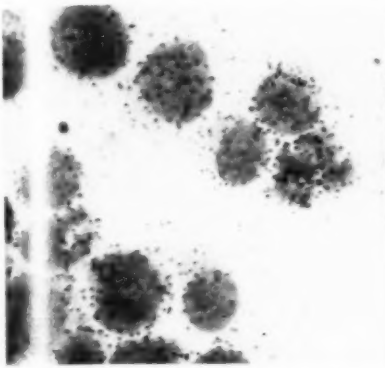

Fig. (No. 565). Photomicrogram of ARG of Ehrlich ascites tumour cells treated in vitro for 30 min with tritiated Compound 1 (0.12 mC and $1 \times 10^{-5}$ M/ml). Magnification: × 1 740.

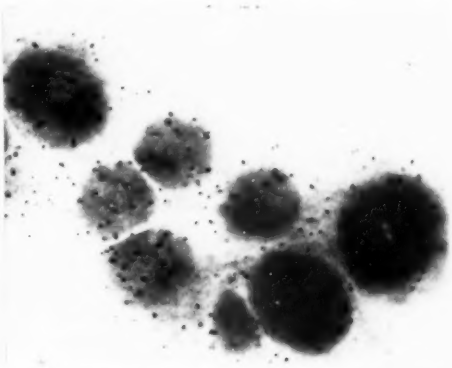

Fig. 2 (No. 713). Photomicrogram of ARG of Ehrlich ascites tumour cells treated in vivo for 30 min with tritiated Compound 1. Final concentration in ascites fluid 0.43 mC and 0.015 mg/ml. Magnification: × 1 740.

*Tissue culture techniques.* These experiments were carried out using three strains of cells, embryonic mouse fibroblasts (E. M. F.), the epithelium of human amnion (H. A.) and the HeLa human uterine cervical carcinoma. The E. M. F. and H. A. cells were used after two passages. Stock cultures were maintained in the usual feeding bottles by the conventional monolayer technique. The nutrient media consisted of (1) Eagle's medium in Hanks BSS 80 parts, human serum, 20 parts, embryonic extract (chick), 10 parts for E. M. F. and (2) Geys BSS 80 parts, bovine serum, 15 parts, lactalbumen hydrolysate, 5 parts for H. A. and HeLa.

For the experimental cultures, the cells were washed in saline free of calcium and magnesium, removed from the bottles by scraping with a 'rubber policeman' (a glass rod with a suitable shaped rubber-covered end) and dispersed by gentle pipetting. After centrifugation, the cells were resuspended in fresh medium and Petri dishes were inoculated with aliquots of the suspension. Each Petri dish contained two (76.2 × 25.4 mm) microscope slides of 0.6 to 0.8 mm thickness. These slides were previously coated with a sterile, photographically inactive gelatine solution recommended for the stripping film technique in autoradiography (Boyd 1955).

It was found in the course of other experiments that even cells grown in a serum-free nutrient fluid settled in monolayers on these slides and that the loss of cells through repeated washing was greatly reduced by their use. The details of the method are as follows: dip grease-free sterile microscope slides in a sterile solution composed of 5 g photographic gelatine, 0.5 g chrome alum, and 1 000 ml water. The solution is sterilized by autoclaving at 5 lb (about 2 268 g) pressure.

The Petri dishes were incubated in air containing $CO_2$ at 37° C for 24 hours. The cultures were selected for the experiment when microscopic examination showed a well established monolayer of cells. The slides were washed three times in warm Ringer's solution to remove serum and covered with the required dose of the appropriate radioactive solution for varying lengths of time at 37° C (see Table 1). After this treatment cultures were again washed in three changes of warm Ringer's solution, fixed in 10 % formalin in BSS for 30 minutes, rinsed in tap water for 10 minutes, then placed in distilled water, and finally used for the autoradiographic studies.

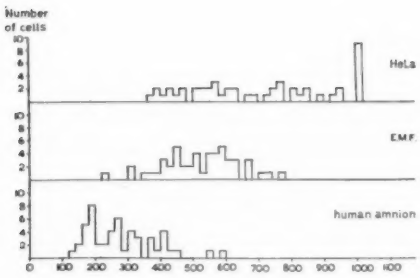


Fig. 3 (Nos 620, 621, 622). Histograms of grain counts per cell in three tissue culture strains treated for 2 min with tritiated Compound 1 (60 mC and $5 \times 10^{-3}$ M). Film exposure time 24 hours.

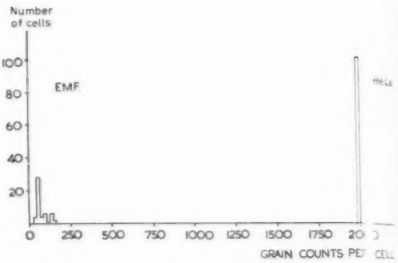


Fig. 4 (Nos 623, 624). Histogram of grain counts per cell of E. M. F. and HeLa treated for 30 min with tritiated Compound 1 (120 $\mu$C and $1 \times 10^{-3}$ M). Film exposure time 72 hours.

*Ehrlich ascites tumour cells.* The Ehrlich carcinoma (hyperdiploid strain of Lettré) is maintained by routine transplantation (0.2 ml every eighth day) into the peritoneal cavity of white mice of our laboratory strain of weight 28 to 35 g. The experimental animals were used on the sixth day after inoculation.

*In vitro experiments.* Ascites fluid in an amount of 0.1 ml was added to 1 ml of the appropriate radioactive solution (see Table 2). All the procedures were carried out at 37° C. The cells were spun down by centrifuging at 1 000 r. p. m. for 2 minutes, the supernatant radioactive solution was discarded and then six smears were made on gelatine coated slides. The residue of cells was washed in 2 ml of warm Ringer's solution, spun down again for 2 minutes and a further six smears made. The smears were dried in air and autoradiographed.

*In vivo experiments.* The mice with Ehrlich ascites tumours were injected with 0.1 ml of tritiated Compound 1, tritiated water or tritiated water containing 'cold' Synkavit intraperitoneally six days after inoculation. The animals were killed at various time intervals, the ascites fluid removed and its volume measured to determine the final radioactivity and concentration of compound in the ascites fluid (see Table 3). Smears were made by the method described above in the in vitro experiments.

*Autoradiographic technique.* Standard procedures were followed (Boyd 1955). Kodak AR 10 stripping film was used for making the autoradiograms (ARG). The exposure time for the films varied from 6 to 144 hours, according to the amount of radioactive material present and was influenced by previous experience.

The cultures and smears covered with the developed stripping film were stained with Ehrlich haematoxylin solution. The percentage of labelled cells was determined by counting 500 cells in at least five different areas of each slide. The results were analysed in the form of histograms of grain counts per cell.

## Results and Discussion

The quantitative results of all the experiments are summarized in Tables 1, 2 and 3. In the experiments using tritiated Compound 1, all the cells were

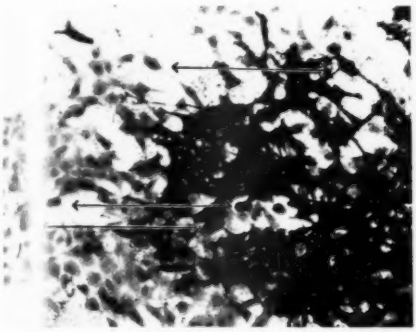

Fig. 5 (No. 624). Low power photomicrogram of ARG of HeLa culture in fig. 4. Cells can be easily identified with their autoradiograms on the slipped film. Magnification: × 100.

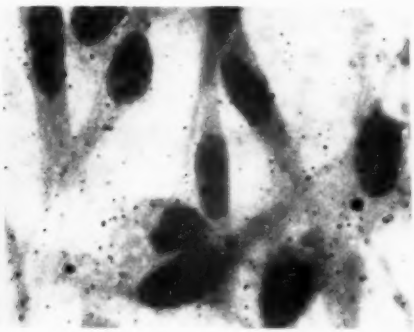

Fig. 6 (No. 623). Photomicrogram of ARG of corresponding E. M. F. in fig. 4. Magnification: × 1 740.

labelled after treatment both in vitro and in vivo. The grain counts per cell increase with increasing specific activity and concentration of the solution and with increasing lengths of treatment. The most surprising feature is the speed with which the tritium of the tritiated Compound 1 is incorporated into the cells. Full labelling was observed in every cell of all the cell types used after two to five minutes treatment with tritiated Compound 1 (Fig. 1). The same behaviour was observed in the in vivo experiments with the Ehrlich ascites tumour cells even at the lowest concentrations used (Fig. 2). This rapid incorporation is consistent with the suggestion that the 'transport form' of tritiated Compound 1 is the unchanged disphosphate (cf. MITCHELL 1960a, DRUCKREY and RAABE 1952) and that active transport into the cells is taking place.

The control experiments (Tables 1, 2 and 3) show that with solution of similar specific activities and concentrations, the passage of the tritium of tritiated water and of tritiated water in the presence of 'cold' Synkavit depends on time. Only after prolonged exposure, a small percentage of cells were labelled in the tissue culture experiments. There was no significant labelling of the Ehrlich ascites tumour cells either in vitro or in vivo. The ordinary 'cold' Synkavit thus had no effect. No further studies of the rate of diffusion of tritiated water into the cells were required in these experiments.

Histograms of the grain counts per cell were drawn out for each experiment. Examples of them are given in Figs 3 and 4. The analysis of all the experiments shows that while all the cells are labelled the rate of uptake of tritiated Compound 1 is different in the three strains of cells in tissue culture. The grain counts for 2 minutes exposure are shown in Fig. 3.

It might be thought that prolonged treatment would increase the relative

uptake in the normal cells. That this is not the case can be seen in Fig 4, which summarizes the results for E. M. F. and HeLa carcinoma cells treated with tritiated Compound 1 for 30 minutes. Under these conditions the grain counts in these two types of cells differ by a factor of more than 100. Figs 5 and 6 are autoradiograms of cells from these experiments.

These short term experiments demonstrate both qualitatively and quantitatively that there is selective uptake of tritiated Compound 1 into proliferating tumour cells.

**Acknowledgements**

I am indebted to Mrs THELMA PRETTEJOHN and Mrs JEANNETTE DAVEY for invaluable technical assistance and to Mr E. W. MITCHELL for photomicrograms. I am grateful to Professor J. S. MITCHELL, F. R. S., for his interest and help.

## SUMMARY

Autoradiographic studies of living normal and malignant cells show a rapid incorporation of the tritium of the tetrasodium 2-methyl-1:4-naphthaquinol (6-T) diphosphate into the cells. A selectivity of uptake by the tumour cells measured by grain counts could be demonstrated.

## ZUSAMMENFASSUNG

Autoradiographische Studien an lebenden, normalen Zellen und an Tumorzellen zeigten, dass obwohl alle Zellen schon nach einer kurzen Zeit mit dem Tritium von dem tetrasodium 2-methyl-1:4-naphthaquinol (6-T) diphosphate markiert waren, konnte eine selective Speicherung in die Tumor-zellen demonstriert werden.

## RÉSUMÉ

L'auteur a étudié par histoautoradiographie l'incorporation du tritium du tétrasodium 2-méthyl-1:4-naphthaquinol (6-T) diphosphate dans les cellules vivantes, normales et cancéreuses. Toutes les cellules sont marquées rapidement, mais la numération des grains montre une fixation sélectives par les cellules tumorales.

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FROM THE DEPARTMENT OF RADIOTHERAPY (DIRECTOR: B. JOLLES), GENERAL HOSPITAL, NORTHAMPTON, AND THE DEPARTMENT OF RADIOTHERAPEUTICS (DIRECTOR: PROF. J. S. MITCHELL), UNIVERSITY OF CAMBRIDGE, ENGLAND.

# INDIRECT RADIATION EFFECTS AND DIFFUSIBLE FACTORS IN IRRADIATED TISSUES (STROMATEX)

by

B. JOLLES, M. REMINGTON and I. SIMON-REUSS

The description of indirect radiation effects is rendered more difficult by the terminology in use. The term 'indirect action of radiation' has two entirely distinct meanings according to whether it is used by radiochemists or radiobiologists and radiotherapists. For the former the indirect action is that on molecules not directly ionized or excited, but which are exchanged as a result of absorption of radiation energy which has excited or ionized solvent molecules, giving products that then act as energy carriers.

By 'indirect radiation effect' in radiotherapy we mean that the effect is one of second reference; the radiation produces a direct effect upon some cellular tissue components, the change in which produces another effect. This effect is an indirect one and is not produced directly by the radiation. Outstandingly the skin is an organ which shows these effects. By custom the term has come to be restricted to the biologic effects produced in a tissue as a result of a radiation-induced injury to the blood supply.

A clarification and a radical modification of both terminology and detailed conceptual significance of terms become imperative on the radiobiologic level, lest the confusion grows deeper and prevents a better understanding of important biologic phenomena. Indeed, the centring of attention on results of radiation effects on blood vessels and the neglect of (1) effects on cells caused

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by stromal effects, (b) the effects on cells and stroma in the irradiated tissues by the neighbouring tissues, (3) the effects on distant tissues by the irradiated tissues and (4) the influence of the distant tissues on the directly irradiated tissues, have acted as a stumbling block in the evaluation of radiobiological and clinical events.

The experimental findings that tumours irradiated in vivo and immediately transplanted may grow while tumours left in situ for a few days after irradiation with the same dose and then transplanted are absorbed, the protective effect in partial body irradiation, the roentgen ray sieve effect, and also numerous clinical observations of distant effects, could be quoted as different aspects of various indirect radiation effects (JOLLES 1953).

Great difficulties are encountered, however, when we try to focus our attention on extracellular events and study the humoral effects, some of which are demonstrable by present-day laboratory methods.

The interplay of normal and irradiation-injured tissues is best exemplified by the course of the radiation reaction of the skin treated through a sieve or grid. The protection afforded by the non-irradiated areas of skin, allowing a higher dose to the exposed areas, probably depends upon the diffusion from the irradiated parts of active products, and on the recovery processes making their way from the normal into the irradiated tissues. These two are intricately connected and their study has to be undertaken under special experimental conditions. The protective role of normal tissues was shown experimentally (JOLLES 1941) when it was found that the dose required to produce a given degree of skin reaction increases as the size of the field diminishes; thus the degree of effect depends on dose times area, and not on the dose alone. When an area was broken up into two or more irradiated sectors, separated by protected areas of skin, the damage produced in each exposed sector was the same as would be produced if the whole area were given a much smaller dose. On the other hand, the degree of reaction of two adjacent areas of skin at varying distances from each other, when compared with that of a single area of skin on a symmetrical site exposed to exactly the same dose (JOLLES 1949, 1950), showed that the reaction increases with the decrease of the separation. This led to the conception of indirect radiation effects attributable to diffusible products in irradiated tissues. Thus, while there is an increase of the skin reaction over two adjacent small areas, a different set of circumstances arises in the case of a large field treated through a multiperforated lead shield in which a reduction in skin reaction is obtained on account of the protective role of skin areas not directly exposed.

The diffusible substances in irradiated tissues and recovery factors making their way from the normal, non-irradiated parts have given rise to much interest and controversy. Although the interdependence of organs and tissues in the animal body should be beyond dispute, in the particular field of radiation reaction, the ideas about events in tissues following irradiation appear to have

en narrowed on account of the confines of the visible skin reaction limited ctly to the directly exposed areas.

These narrow limits have been widened when it was shown — by a series experiments on grafting of normal rabbit's skin into an irradiated bed and diated skin into a normal bed — that radiation effects become visible side the irradiated area when some of the recovery and repair processes king their way from the non-irradiated parts are engaged or neutralized, only by the radiation injury-induced products, but also by the surgical uma and scarring processes (JOLLES & GREENING 1958).

In our previous papers, experimental studies on the indirect radiation effects re reported and also the work of other authors reviewed. The study of these tors has passed the phase of demonstration, and the actual collection of logically effective factors and the study of effects on mitosis in tissue cultures e been undertaken. These studies are described in the present paper.

The different processes forming together the phenomenon of inflammation, it due to chemical or thermal stimuli or to ionizing radiations, are deeply volved in the radiation reaction. The separation of the injury and recovery processes is one of the problems of radiobiology which has to be studied before any progress is made in the clinical and experimental fields.

*Comments on methods.* Since an increased capillary permeability is prominent also in the radiation reaction, it was thought advisable to follow in the course of the study the technique used by MILES and WILHELM (1955) in their investigations of the intermediary factors causing increased vascular permeability in inflammation. These authors used a vital dye (Pontamine Sky Blue) injected intravenously prior to injury, and the degree of 'blueing' of the affected skin area served to demonstrate the degree of increased capillary permeability.

This would in a certain measure give a clue as to the optimum time for collection of some of these factors. By injecting a vital dye (Pontamine Sky Blue, 60 mg/kg, i.e. 1.2 ml/kg of a 5 % solution in 0.425 % saline) into the marginal ear vein of rabbits whose depilated flanks were exposed over small areas to doses of roentgen rays generated at 100 kV (HVL 0.11 mm Cu) it was possible to determine the time of the maximum of a well defined blue flush over the irradiated area. This appeared in preliminary experiments within 2 hours after irradiation and lasted up to 24 hours.

Experiments were then carried out on 26 stock farm bred albino rabbits, each serving as a test and control animal. Areas 2 cm in diameter on the depilated flanks were exposed to a dose of roentgen rays and at varying intervals of time after the irradiation the vital dye was injected. The time intervals tested were 1, 2, 3, 4, 6, 7, 9, 15, 16, 18, 20, 21 and 23 days, and the most intense blueing was consistently noted at intervals of 1, 9 and 16 days after irradiation, with an occasional but less consistent reaction at 4 days and 20 to 2 days (Tables 1 and 2).

**Table 1**

*Diffusible substances in irradiated tissues in 'time course' experiments*

| Experiment No. | Dose in 'r' | Days on which areas were irradiated (intervals between irradiation and injection of Pontamine Sky Blue) | Days on which reaction occurred (intervals between irradiation and the most intense blueing reaction) |
|---|---|---|---|
| 21 | 1 200 | 1 4 9 16 | 1 4 16 |
| 22 | 1 200 | do | 1 9 16 |
| 23 | 1 200 | do | 1 9 16 |
| 24 | 1 200 | do | 1 16 |
| 25 | 1 200 | do | Negative |
| 26 | 1 200 | do | 1 4 16 |
| 7 | 2 000 | 1 3 6 9 15 20 23 | 1 9 20 23 |
| 8 | 2 000 | do | 9 15 |
| 11 | 2 000 | 1 3 7 | 1 |
| 12 | 2 000 | do | 1 |
| 15 | 2 000 | 1 2 4 18 21 | Negative |
| 16 | 2 000 | do | Negative |

**Table 2**

*Summary of the results of time-course experiments which are shown in detail in Table 1*

| Time interval in days | No. of rabbits irradiated | No. of rabbits with positive reaction | Percentage of positive reactions |
|---|---|---|---|
| 1 | 12 | 8 | 66 % |
| 4 | 8 | 2 | 25 % |
| 9 | 8 | 4 | 50 % |
| 16 | 6 | 5 | 83 % |
| 20—24 | 6 | 2 | 33 % |

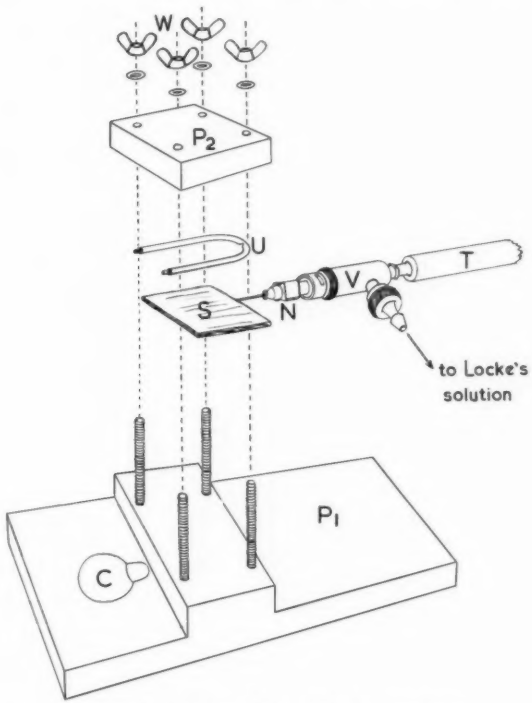
After having so established the optimum time for collection, it was decided to obtain samples from irradiated and control areas of skin of the same rabbit at 10, 30, 80 and 90 min, and 1, 9 and 16 days after irradiation.

## Technique of 'stromatex' collection

In naming this factor, although its chemical nature is not yet ascertained, 'stromatex' was chosen because an extravascular, stromal origin is surmised (JOLLES 1960).

The technique of MILES and WILHELM (1958) was used here again. The irradiated and control areas of skin are removed from the animal immediately after killing and are perfused with Locke's solution, using the apparatus illustrated in the figure on opposite page.

The skin (S) is placed, subcutaneous surface upwards, on the lower perspex




Schematic drawing of the perfusion apparatus.

block ($P_1$) and a No 26 gauge needle (N) inserted into the skin below the dermis. A rubber covered U-piece (U) notched at the mid-point to accommodate the thickness of the needle is placed on the skin, covered by the second perspex block ($P_2$) and the whole clamped together tightly by means of wing-nuts (W). A three-way valve (V) is attached to the needle and a tuberculin type 1 ml syringe (T) and a reservoir of Locke's solution connected to the valve are used. Locke's solution is drawn up into the syringe and injected under pressure of 3 to 4 atmospheres into the skin, where it flows to the only free edge, the direction of the flow being limited by the U-piece. The perfusate, a fairly limpid opalescent fluid, containing, apart from Locke's solution and tissue fluids, presumably some active substances in the case of irradiated skin, collects in a well (C) in the lower perspex block below the free edge of the skin. Forty international units of hyaluronidase are added to the first 0.5 ml of Locke's solution to facilitate perfusion.

**Table 3**

*Inhibition of mitosis by 'stromatex'*

| | Experiment No. | Number of cells in mitosis as % of untreated controls | | | | |
|---|---|---|---|---|---|---|
| | | Prophase | Metaphase | Anaphase | Telophase | Total |
| Chick fibroblast cultures + perfusates from normal skin | A | 98.9 | 102.5 | 91.5 | 97.5 | 99.0 |
| | $B_1$ | 96.2 | 88.9 | 94.3 | 94.1 | 93.0 |
| | $B_2$ | 96.2 | 88.9 | 94.3 | 94.1 | 93.0 |
| | C | 105.8 | 89.9 | 125.8 | 101.6 | 100.0 |
| Fibroblast cultures + perfusates from skin irradiated 30 minutes previously | A | 54.0 | 63.0 | 50.8 | 51.9 | 56.0 |
| | $B_1$ | 45.3 | 48.7 | 41.4 | 38.3 | 43.2 |
| | $B_2$ | 85.6 | 77.8 | 58.4 | 106.6 | 89.6 |
| | C | 60.5 | 49.5 | 91.5 | 48.5 | 53.3 |
| | D | 52.9 | 48.5 | 45.9 | 45.8 | 47.7 |

The perfusates from irradiated and non-irradiated areas of skin were subjected to various tests. One of these was upon mitosis in chick and mouse fibroblasts and this was undertaken (I.S.-R.) at the Radiotherapeutic Centre in Cambridge.

Chick fibroblasts were cultured by the hanging drop method in plasma and embryonic extract, with or without perfusion fluid. The cultures were incubated for 24 hours, fixed and stained, and total mitosis counted in controls and in treated cultures. The perfusates obtained 24 hours and 16 days after irradiation showed no definite effect on mitosis. Perfusates obtained 10 min after irradiation produced some cytological abnormalities in mitoses, e.g. cell oedema, clumped chromosomes in metaphase and some sticky anaphase, but no mitotic inhibition. With perfusates obtained 30, 80 and 90 min after irradiation, however, a definite mitotic inhibition was observed and also nuclear abnormalities including accumulation of cells in meta- and anaphase, and clumped and fragmented chromosomes (Table 3).

Mouse fibroblasts were cultured on coverslips in Petri dishes by the monolayer technique and the perfusates incorporated into the supernatant nutrient fluid. Coverslips were removed at 24, 48 and 72 hours and mitosis counted on an average of a 1 000 resting cells. No effect on mitosis could be found with perfusates from skin irradiated 1.5 hours, 24 hours and 16 days previously. A slight effect on resting cells was seen at 24 hours, but by 72 hours all cells had recovered completely.

In a further series of experiments, perfusates have been injected into growing Crocker (S. 180) sarcoma in mice. The 30 min perfusate seems to have a slight inhibitory effect on growth, but so far the results are not numerous enough to form a definite opinion and the work is being continued (JOLLES and REMINGTON 1960).

Chemical studies, paper chromatography, electrophoresis, and others, are being done. The lack of an inhibitory effect of the perfusates from tissues ir-

r[illegible]iated more than 90 min previously is not surprising when one assumes that t[illegible] biologically active radiation injury products are either rapidly transported a[illegible]y from the site of their formation or are neutralized by the recovery factors o[illegible]he surrounding non-irradiated neighbourhood, and by the intrinsic re-s[illegible]ative power of the irradiated part of the skin, not completely exhausted at t[illegible]e dose levels. Observations made in grafting experiments showed that t[illegible]e was no marked difference between the fate of grafts kept in saline for 10 m[illegible] and those kept for 60 min after irradiation. This led to the belief that the d[illegible]usion of 'toxic' substances took place over a period longer than one hour. T[illegible] more recent series of experiments shows that the activity increases from 1[illegible]o 30 min, after which it decreases up to 90 min.

[illegible]ome of these experimental findings (e.g. the optimum time for the collection of [illegible]he most intensely active perfusates and the length of time of their effectiveness) throw light on the time when recovery factors in irradiated tissues manifest themselves after radiation injury and explain the minimal effectiveness of diffusible substances on tumour not directly exposed to radiation.

## SUMMARY

The interplay of irradiated and normal tissues and the role of diffusible substances in the radiation-induced inflammatory reaction are discussed. The time at which the maximum yield of biologically active material is obtained from irradiated skin has been established in experiments with vital dyes injected into the animals at varying intervals after irradiation. The technique of perfusion of rabbits' skin is described and the results of tests on mitotic inhibition in tissue cultures of chick and mouse fibroblasts by perfusates from irradiated skin ('stromatex') and normal skin of the same animals are given.

## ZUSAMMENFASSUNG

Die Wechselwirkung zwischen normalen und bestrahlten Geweben und die Rolle diffusibler Substanzen in der durch die Bestrahlung verursachten Entzündung werden besprochen. Der Zeitpunkt, an dem man ein maximales Sammeln biologisch wirksamen Materiales in bestrahlter Haut erhält, wurde in Tierexperimenten festgestellt, in denen man Vitalfarben mit wechselnden Zeitabständen nach der Bestrahlung injizierte. Die Methode der Perfusion der Kaninchenhaut wird geschildert und die Ergebnisse der Untersuchungen über die Inhibierung der Mitose in Gewebekulturen von Hühner- und Mäuse-Fibroblasten durch die Perfusionflüssigkeit von bestrahltem 'Stromatex' und normaler Haut werden beschrieben.

## RÉSUMÉ

Les auteurs étudient l'influence réciproque entre les tissus irradiés et les tissus normaux, ainsi que le rôle des substances diffusibles dans la réaction inflammatoire due à l'irradiation. Le moment où la peau irradiée fournit le plus de substance biologiquement active a été déterminé expérimentalement par des injections de colorants vitaux à des animaux, à différents intervalles après l'irradiation.

La technique de perfusion de la peau de lapins est décrite; les auteurs donnent les résultats de tests d'inhibition mitotique sur des cultures de tissus de fibroblastes de poussin et de souris sous l'effect de liquide de perfusion de peau irradiée 'stromatex' et de peau normale de ces mêmes animaux.

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# GENETIC HAZARDS OF RADIATION TO MAN
## PART I

by

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Because greatly varying statements and inferences are forthcoming on the seriousness or the triviality of the genetic risks from radiation, it is proposed here critically to review the available evidence. In particular, it shall be indicated that a new analysis of the data gathered by the official U. S. Commission of enquiry in Hiroshima and Nagasaki compels one to question its generally negative conclusions. An attempt shall be made to estimate the magnitude of the hazard which the present and the possible future levels of ionizing radiation pose to the posterity of man.

### Experimental production of genetic effects

MULLER (1927) was the first to demonstrate the 'artificial transmutation of the gene' on *Drosophila* by means of roentgen rays. The production of hereditary effects by ionizing radiation has since been confirmed in a number of species; the large amount of information on this question has been usefully reviewed by MULLER (1955) and W. L. RUSSELL (1954). Induced changes in the hereditary material may consist of major chromosome aberrations or breaks (giving rise to inversions, deletions, duplications and translocations) or to 'point' or gene mutations (believed to represent molecular alterations within individual genes). All such induced changes are similar to the mutations which occur spontaneously and are due in part, it is thought, to natural radio-activity, both external (ground, cosmic rays) and internal (potassium 40 in tissues). The frequency of these changes may be increased by a variety of chemical mutagens and physical changes, as well as by ionizing radiations.

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X': an X-chromosome bearing a (lethal) major aberration.
The abnormal chromosome from the father can be transmitted only to a female conceptus, which does not survive.
(A recessive mutant gene would not be lethal in the heterozygous female embryo.)

X": an X-chromosome bearing a (lethal) mutant gene.
The mutant gene from the mother is lethal to a hemizygous male, but if recessive does not affect a heterozygous female.
(A dominant chromosomal change would be lethal for concepti of both sexes.)

Fig. 1. Sex-ratio effects ('sex-linked lethals'). According to the present genetic conception, the proportion of males in the progeny may be increased by massive irradiation of the father's gonads and decreased by irradiation of the mother's.

It is agreed that most, if not all, new mutations are deleterious. In plants, much fewer than 1 % of radiation-induced mutants are found to be useful. In insects, a few induced mutations confer some advantage, but only to completely inbred populations — a situation quite in contrast with the human condition (NEWCOMBE 1960). According to DOBZHANSKY (1957): 'if anything, radiation-induced mutants are more destructive than the spontaneous ones. As far as genetic effects are concerned, the only safe dose of high-energy radiation is no radiation'.

As observed in mammals, mostly the mouse, the effects of radiation-induced mutations may be:

(1) Dominant: lethals, seen as reduced litter size, or non-lethals, evidenced as offspring which are malformed or diminished in vitality or life-span; semisterility, resulting from translocation, is seen one generation later as a reduction of litters by one-half and is handed down to subsequent generations (PAULA HERTWIG 1940).

(2) Recessive mutations are demonstrated by mating the offspring of exposed males to females known to be (heterozygous) carriers of certain traits (W. L. RUSSELL 1954; CARTER et coll. 1956).

(3) Mutations involving sex-chromosomes may be reflected by a change in the sex ratio of the offspring (sex-linked lethals, see Fig. 1).

The production of mutations depends not only on the dosage of radiation (as reviewed in the next paragraph), but also on the kind of cells irradiated. SCHINZ & SLOTOPOLSKY (1925) were the first to show, with testicular irradiation, that 'reserve spermatogonia' are less sensitive than active spermatogonia. Likewise, the sensitivity of oöcytes, as measured by the induction of dominant lethals, is greatest when the interval between irradiation and mating is the shortest (L. B. RUSSELL & SPEAR 1955). However, as will be seen presently, immature germ-line cells are not immune to mutagenic influence. Mutations occurring in mature gametes are demonstrable for a short time only, because after a period of a few weeks (during which the subject becomes temporarily

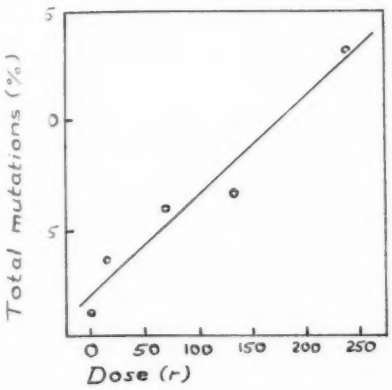


Fig. 2. Dose-effect relationship in the production of mutations (dominant and recessive) in mice by chronic exposure to roentgen rays. As little as 13 r manifestly accelerates the occurrence of mutations. A least-squares regression line has been drawn. The regression coefficient + 0.00448 % per roentgen is highly significantly different from zero ($t = 16.55$; $p < 0.01$). The linear correlation ($r = 0.955$) is also significant ($p < 0.02$). Based on data from CHARLES (1950).

sterile if the dose of radiation was massive enough), the injured gametes are replaced by new ones freshly matured from spermatogonia or oögonia. For that reason, a person accidentally exposed to a significant dose of radiation could, by refraining from procreation for a few months, prevent the genetic effects most likely to be visible in offspring conceived early. But that precaution would not remove the probability that mutations induced in germ cells at an early gonial stage of development, already observed by MULLER (1927), would be transmitted to many daughter-cells, eventually to many functional gametes and to the germ plasm of offspring. These changes would be permanent, as indeed they are in experimental animals where the magnitude of effect is found to be the same in offspring conceived early or late following the sterile period; that is, the mutation rate does not decrease with the time elapsed since exposure (W. L. RUSSELL). Such changes might, or might not, be evident in the first generation, but could cause an increase of disease or disability in future generations. In point of fact, as MULLER (1955) notes, the less harmful a mutant, the greater the number of individuals it affects, inasmuch as reproduction remains possible. Thus the total measure of mutational harm is the number of mutations produced, of whatever degree of harmfulness, rather than the damage to any one individual or generation. In a population which, like the human, is not inbred, the generation showing the greatest effect of a mutation should be the first filial one; but the total effect in the posterity, over an indefinite number of generations, is still greater (CROW 1958). This point shall be illustrated in relation to the foreseeable effects of radiation upon descendants of man (see Part II).

Most classical work on the induction of mutations in mammals involved doses ranging from 200 to 1 600 r (HERTWIG; HENSON; W. L. RUSSELL). What is most important in relation to human hazards is the significance of small amounts of radiant energy. It is a classical finding in *Drosophila* and in lower life forms (bacteria, bacteriophage) that the relation between dose and

effect is 'linear' and cumulative; a given dose of radiation produces the same total effect, whether given at low intensity over a long period or in one large dose, and the linearity of the dose-effect relation holds so constantly that no lower limit, or threshold, can be set for the mutagenic effect of roentgen rays (MULLER 1954). Indeed, MARCOVICH (1956), working with the lysogenic system of *E. coli* K 12, sets a minimum effective dose below 1 r and postulates that a single photon of high-energy radiation possesses mutagenic action.

Although one frequently reads that the linearity of the dose-effect relation has not been demonstrated in low-dose irradiation of mammals, it is possible to show from CHARLES' (1950) experiments on the mouse a highly significant mutagenic action, with a significantly linear dose-effect relation ranging from a minimum dose of 13 r (Fig. 2). In one recent study, however, on spermatogonial mutations in mice (revealed by mating after the sterile period), preliminary data indicate that chronic low-intensity gamma radiation may produce a lower induced mutation rate per unit of radiation than acute subjection to high-intensity roentgen rays (W. L. RUSSELL et coll. 1958). This 'dose-rate' effect, if confirmed by significant data, would modify the concept of a linear dose-effect relationship, but need not invalidate the no-threshold idea. Rather, it could be explained by the presence in immature germ cells of something which affords protection or recovery from mutation at low-intensity exposure, but is inhibited at high intensities of radiation. It is also of interest to note in the same report that whereas '... chronic gamma irradiation of oöcytes gave mutation rates lower than those from acute x-irradiation of spermatogonia, acute irradiation of oöcytes is at least as effective as acute irradiation of spermatogonia'. Thus women should be expected to be as vulnerable as men to the genetic hazards of massive irradiation.

A number of writers have questioned the validity of extrapolating from the results of animal experimentation to man. Indeed, one enthusiastic defender of so-called 'stimulating' doses of roentgen rays to the ovaries for the correction of infertility (see Part II) has complained that 'too many doctors have been scared ... by fruit flies, mice, and men' (ISRAEL 1952). However, one cannot discount the near-certainty that the genetic effect of radiation, which has been proved in a number of species, is a general biological law to which man is no exception. One could repeat here what RUGH (1958) says in relation to foetal lesions: every effect believed to be due to radiation in the human can be produced at will in the experimental animal. Thus it seems that the only limitation to inferences from the animal to the human lies in the question of dosage. In this connection it is of interest to note, with W. L. RUSSELL (1954), that dominant effects are induced by roentgen rays at a much higher rate in mice (with 20 haploid chromosomes) than in *Drosophila* (with 4) and that 'it is reasonable to suppose that, with 24 chromosomes in man, the rate in man might be somewhat higher than in the mouse'. That the count of chromosomes in the haploid human cell is now known to be only 23 does not, of course, invalidate this thinking.

**Table 1**

*Studies of radiation mutagenesis in man. Mutation may be produced in man by doses of ionizing radiation in the higher diagnostic range.*

| Effect | Worker | Kind of rays | Dose | Significance[3] |
|---|---|---|---|---|
| Congenital malformations in offspring of physicians with and without occupational exposure | MACHT & LAWRENCE 1955 | Mostly roentgen chronic | 25 r?[1] | $p = 0.012$ for all defects: $p = 0.02$ for heart dis. |
| | CROW 1955 | Same | Same? | None |
| | TENTSHOV & coll. 1959 | Same | Same? | $p = 0.02$ |
| Sex-ratio changes in offspring of persons undergoing radiotherapy | TURPIN, LEJEUNE & RETHORÉ 1956 | Roentgen or radium therapy | Mean 721 r skin dose | $p < 0.02$ for mean dose 1 730 r |
| Abortions in same | *idem, in* U. N. Report, p. 195 | Same | Same | $p < 0.01$ |
| Chromosome aberrations in somatic cells in culture | BENDER[2] 1957 | Roentgen acute | 25 r | $p < 0.001$ |

[1] Estimated as 2.5 r/year (average gonad dose for radiologists: see MRC Report 1956, p. 119) for a period of 10 years between usual age of commencing clinical work (20 yrs) and mean age of fathers at conception of offspring (30 yrs).

[2] See also Part II, Table 5 (doubling dose 3.3 r).

[3] Based on statistical analysis of author's data (comparison with controls).

It remains unfortunately true that the exact determination of the genetic hazard of radiation to man is fraught with many difficulties, which have recently been reviewed by NEWCOMBE (1960) and are further analyzed below. HALDANE (1958) sadly points out that the experimental work on mice which could give us most of the answers might have been done already for but a fraction of the cost of one H-bomb. The remainder of this study examines the direct evidence pertaining to the mutagenic effect of radiation in man.

## Clinical studies of genetic effects in man

Planned experiments similar to those just described have obviously not been possible with human beings. Apart from the war-time exposure of large populations to radiation, which we shall study later, one has to be content with surveys of human subjects who, through occupational exposure or for therapeutic reasons, have been subjected to ionizing radiation. One such study, conducted in the U. S. A. by MACHT and LAWRENCE (1955), compared the incidence of twins, foetal deaths and congenital defects in the offspring of two groups of physicians, one (consisting mostly of radiologists) with occu-

pational exposure to roentgen rays, the other without such exposure. The total number of offspring surveyed was 9 945. The significant differences found, which have been summarized in Table 1, were a higher incidence of congenital defects in the exposed group and a significantly higher ratio of males to females in the malformed offspring. Among the defects, the categories showing the greatest differences were that of congenital heart and great-vessel anomalies and, surprisingly, that of haemolytic disease of the newborn. 'Neoplasms', including naevi and cysts, were more frequent, but not significantly, in the exposed group. The differences retained their significance when an adjustment was introduced for the slight difference in mean parity (birth order) between groups. However, doubts have been expressed (ABCC Report 1956, and U.N. Report 1958) on the reliability of this survey, which was conducted through mail questionnaires with unequal response in the two groups. A similar study, reported by CROW (1955), bore on a comparison of about one-quarter as many offspring of radiologists and of pathologists, and failed to show any difference in the numbers of foetal and neo-natal deaths; sex-ratio and malformations were not considered. A recent report from Bulgaria (cf. Table 1) gives results similar to those of MACHT and LAWRENCE.

In France, TURPIN, LEJEUNE & RETHORÉ (1956) have studied the indicators of genetic change in 614 offspring of persons having undergone radiotherapy involving the pelvic area. The first data published were those on sex-ratio. According to hypothesis (Fig. 1), induced sex-linked mutations tend to increase the masculinity of the offspring of exposed men (or, at least, of those exposed heavily: see p. 75) and to reduce the masculinity of the offspring of exposed women. Such an effect has been shown (Table 1): a significant difference exists in the sex-ratio of the offspring of treated mothers and that of fathers having received more than 1 000 r (skin dose), whereas the offspring of the same parents, born before exposure, had not differed significantly in sex-ratio. The data on abortions, stillbirths and congenital malformations are available only in sketchy form (see U.N. Report 1958, p. 195); in all 3 categories, there is a higher rate of wastage in the progeny of a parent exposed before conception than in that born to the same parent prior to therapy. The difference for the abortion rate only is highly significant, in relation to both paternal and maternal exposure. It is too marked to be explained by the obviously higher parity in post-therapy offspring than in the pre-therapy group.

Relevant to the possible ill-effects of small amounts of radiation on human populations is an epidemiological survey conducted in the state of New York (GENTRY et coll. 1959) where the incidence of congenital malformation was found to be significantly higher in certain townships with high levels of natural radiation from the ground (granite outcrops, silicon-rich rocks) than in other locations with low natural radiation. Although the possibility is not excluded of other factors varying between mountainous regions and plains (standard

of ing and nutrition, oxygen tension available to the foetus), it seems re: nable to accept that the difference in incidence of malformation is ca: lly related to the intensity of background radiation. Another study to el: late this point has been initiated on the population inhabiting the th: m-rich monazite sands of Kerala, India ('Effect of radiation on human he: ity' 1957); no results are available as yet. WESLEY (1960) has shown tha the death-certificate incidence of congenital malformation in many cou ries varies significantly with the geomagnetic latitude, to which is related the osmic-ray energy flux. It must be noted, however, that there is not a goo agreement between available determinations of total background radiati in certain parts of the earth's surface and the intensity of cosmic rays; on e other hand, there exist from country to country too great differences in ving conditions, degree of medical information, prevalence of autopsy and ocal concepts of what constitutes malformation (even when the W. H. O. clas ification is observed) for this relationship to be convincing. In any case, should the teratogenic effect of background radiation be documented more firmly, one would not be certain whether it were mediated through the genes only, or involved as well an influence on the embryo after conception.

## Atomic bomb studies of genetic effects in man

The most massive exposure of human persons to ionizing radiations took place in August, 1945, in the Japanese cities of Hiroshima and Nagasaki. The desirability of detecting any genetic effects of such exposure was recognised by the U. S. A.-sponsored Atomic Bomb Casualty Commission (ABCC Report 1956). To this end, between February 1948 and February 1954, about 65 000 births in both cities were studied; the results were reported in great detail by NEEL, SCHULL and many others. In this publication, referred to henceforth as the 'ABCC Report', possible genetic effects are studied in relation to maternal age, parity, maternal state of health, parents' socio-economic status, as well as to exposure of the parents to the bomb. For the latter factor, the births (after eliminations of multiple births and of those from consanguineous unions) are distributed in a number of 'exposure cells' according to a system of grades, as follows for each parent:

| Exposure category | Defined as: | Estimated dose (rep) |
|---|---|---|
| 1 | Absent from either city at time of explosion ..... | 0 |
| 2 | Exposed lightly (more than 3 000 metres from hypocentre, or nearer but with heavy shielding) ..... | 5 to 10 |
| 3 4 | Exposed moderately (various combinations of distance and shielding) ..... | 50 to 150 |
| 5 | Exposed heavily (within 3 000 metres and with major radiation-sickness symptoms: epilation, petechiae, gingivitis) ..... | 200 to 500 |

Among the various factors which may influence the incidence of reproductive accidents, the maternal state of health and the parents' socio-economic status did not differ in these categories. The relationship between exposure and maternal age, as well as parity, is discussed below.

The general conclusion of the ABCC Report is that no consistent genetic effects of radiation could be shown in a study of the sex-ratio and of the incidence of malformations at birth, stillbirths and neo-natal deaths. The authors add that only slight effects could have been detected in any case, and that their conclusions should not be interpreted as meaning that induced genetic factors were not, in fact, operating in the population. The first point is important: the near impossibility of showing marked effects in an unplanned experiment such as the nuclear bombing of even a large population has been stressed by PENROSE (1956). The large number of concomitantly varying factors, and the occurrence of reproductive accidents due to causes other than radiation, must needs obscure the small portion of the total induced genetic damage which could be detected in a partial survey of the first filial generation.

It is felt, however, that the methods of analysis employed in the ABCC Report are open to a certain amount of review. Of their rather complicated system of correcting the distance from the hypocentre by the amount of shielding (light, moderate or heavy) which may have protected the exposed person, the authors of the report themselves admit (p. 44) that it is 'conservative' and has the effect of relegating to class 2 (exposed lightly) many individuals who must have suffered appreciable radiation effects. In a preliminary publication, NEEL and SCHULL (1953) had indicated a significant effect of maternal exposure (without shielding correction) on the sex-ratio; in 1956 they state that with the 'improved' system of exposure categories, this effect is no longer significant. A correction for shielding has not seemed necessary in other studies in which the incidence of microcephaly following prenatal exposure (MILLER et coll. 1956), and that of leukaemia in all survivors (YAMAWAKI 1953, MOLONEY & KASTENBAUM 1955) both correlate well with the distance from the hypocentre; one may thus well ask if the shielding correction constitutes an improvement for any reason other than that it affords a more reassuring view of the long-term effects of atomic bombing. Be that as it may, this correction cannot be removed from the analysis of the data as presented in the ABCC Report and we must accept a certain amount of dilution of the lightly-exposed group.

In almost all of their analyses, the authors of the ABCC Report measure the statistical weight of the exposure of one parent, without taking into account whether the other parent was exposed or not: thus, in comparing the groups of births from unexposed mothers with those from exposed mothers, in each group the fathers had been exposed to various degrees in some cases and unexposed in others. A dilution of exposure is thereby introduced into the

**Table 2**

*Results of the atomic bomb genetic study when re-analyzed for the effect of one-parent exposure. Significant genetic effects in the bombed population can be ascribed to irradiation in relation both to paternal exposure (neonatal deaths) and to maternal exposure (sex-ratio and stillbirths). The ensemble of the data (total loss) is significant even when paternal exposure alone is considered. The unexposed population is not considered. Based on data in the ABCC Report.*

| | | | Births | Index cases | | $\chi^2$ | df | P |
|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | | | |
| Sex ratio (male to total) | Father only exposed | lightly | 3 670 | 1 892 | 51.55 | 0.68 | 1 | — |
| | | heavily | 1 373 | 726 | 52.88 | | | |
| | Mother only exposed | lightly | 14 684 | 7 681 | 52.31 | 6.01 | 1 | [2] $< 0.02$ |
| | | heavily | 4 608 | 2 324 | 50.43 | | | |
| Malformations at birth | Father only exposed | lightly | 3 670 | 28 | 0.763 | 3.35 | 1 | — |
| | | heavily | 1 373 | 18 | 1.311 | | | |
| | Mother only exposed | lightly | 14 684 | 121 | 0.824 | 0.30 | 1 | — |
| | | heavily | 4 608 | 42 | 0.911 | | | |
| Stillbirths | Father only exposed | lightly | 3 629 | 57 | 1.570 | 0.25 | 1 | — |
| | | heavily | 1 354 | 24 | 1.772 | | | |
| | Mother only exposed | lightly | 14 547 | 216 | 1.484 | 4.67 | 1 | [2] $\sim 0.03$ |
| | | heavily | 4 561 | 89 | 1.951 | | | |
| Neonatal deaths | Father only exposed | lightly | 3 572 | 49 | 1.372 | 4.11 | 1 | [2] $< 0.05$ |
| | | heavily | 1 330 | 29 | 2.180 | | | |
| | Mother only exposed | lightly | 14 331 | 217 | 1.514 | [3](0.16) | 1 | — |
| | | heavily | 4 472 | 64 | 1.431 | | | |
| Pooled paternal and maternal exposure | Total loss: | | | | | 19.21 | 7 | [1] $< 0.01$ |
| | Total loss, excluding sex-ratio loss: | | | | | 12.52 | 5 | [2] $< 0.03$ |
| Paternal exposure only | Total loss: | | | | | 8.39 | 3 | [2] $< 0.05$ |
| | Total loss, excluding sex-ratio loss: | | | | | 7.71 | 2 | [2] $< 0.03$ |

[1] A highly significant difference.

[2] A significant difference.

[3] An insignificant difference in a direction contrary to the genetic hypothesis; this contribution to $\chi^2$ is subtracted from the total.

unexposed groups, and vice versa; this is undesirable, in view of the genetic hypothesis that germ cells of both sexes are subject to mutagenic influences, and especially serious in the case of the sex ratio, upon which exposure of father and mother are expected to act in opposite directions (Fig. 1). Fortunately, the data in the report are presented in such a way that this objection can be removed, and in the next paragraph a consideration shall be given to the apparent effect of the separate exposure of each parent. Further, it is necessary to agree with NEEL and SCHULL that comparisons between groups com-

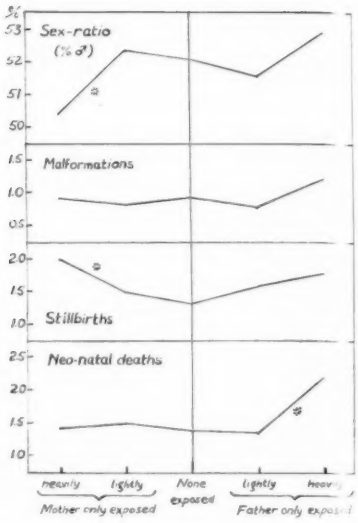


Fig. 3. Genetic effects of exposure to atomic detonations in Hiroshima and Nagasaki shown by a comparison of the offspring after light or heavy exposure of one parent (method of analysis discussed in text); see also Table 2. The parameters belonging to the unexposed population are shown in the mid-line of the figure but are not taken into account in the discussion, for reasons given in the text. Statistically significant differences ($p < 0.05$) are shown by asterisks. Based on new analysis of data from NEEL, SCHULL et coll. (Nat. Res. Counc. Publ. 461, 1956).

prising both unexposed and exposed are faulty, since the unexposed population, having been outside of either city at the time of the bombing, is a different population, most of which immigrated into the cities later. The validity of this doubt can be illustrated by the significantly different mean maternal age and parity in the unexposed and exposed, which have been calculated from the data in the report and are given elsewhere (DE BELLEFEUILLE 1960).

A set of comparisons is therefore in order between pairs of groups, each group of every pair differing only in that one parent has been exposed lightly, or heavily, the other parent having been unexposed in every case. The lightly-exposed parent (exposure category 2) can be considered, for the present purpose, a virtually unexposed control, the dose of radiation being estimated in this class at 5 to 10 rep. The group of heavily-exposed parents shall consist of all the other exposed, moderately or heavily according to the classes used in the report (categories 3, 4, 5 pooled). The validity of this type of comparison is shown by the fact that mean maternal age does not differ significantly in relation to light or heavy one-parent exposure, either paternal or maternal (DE BELLEFEUILLE 1960). In contrast, there is a marked difference in mean parity (birth order) in relation to both 'pure' paternal and maternal exposure; this, as discussed below, may well, in itself, reflect a genetic effect. One may safely assume, therefore, that one is dealing with equivalent populations, and glance at the result of the comparisons (Table 2). There is a well-marked trend in the direction of the genetic hypothesis in 7 of 8 comparisons involving one-parent exposure, and in 3 the difference is statistically significant: or

r ernal exposure toward the sex-ratio and the incidence of stillbirths, and p rnal exposure toward the incidence of neo-natal deaths. The same data a represented graphically in Fig. 3 where the statistically significant effects a marked by asterisks.

he comparisons outlined in Table 2 require some comment.

*Sex-ratio.* This significant result of maternal exposure has been shown in ie ABCC Report (p. 92); it is, in fact, the only computation involving 'o -parent' exposure to be found therein. The effect of paternal exposure, w h is in the opposite direction, though not significant statistically, is in ac rd with the genetic theory (Fig. 1). More precisely, as discussed by L UNE and TURPIN (1957), male births are expected to regress from light pa rnal irradiation, and to increase from massive exposure, major aberrations in ie X-chromosome being postulated in the latter case. As seen in Fig. 3, ju these tendencies are suggested in the atomic-bombed population.

*Malformation at birth.* The trends are not significant statistically. Some po ible reasons for this suggest themselves. What would seem at first sight to e a serious lack in the data collected is the omission of congenital heart an great-vessel disease, decided upon in the ABCC study because of unreliability of the diagnosis of such lesions at birth. Although granting the latter point, one might think, in view of the results of other studies, that this exclusion, amounting to 97 cases, would explain in part the negative result obtained. However, SCHULL (1959) reports '... an incidence of congenital heart disease of 1.60/1 000 births if the parents were unexposed, 1.52/1 000 births if one or both parents were lightly exposed (radiation category 2), and 0.98/1 000 if one or both parents were moderately or heavily exposed (radiation categories 3, 4, or 5)'. Although the incidence in relation to one-parent exposure cannot be computed from these data, it is almost certain that no marked effect would emerge. Rather, then, the absence of a significant effect of parental exposure must come from the nature of the material surveyed. We have seen that while the mothers who were exposed lightly did not differ significantly in mean age from those who were exposed heavily, the latter showed a notably lower mean parity. This means, in effect, that the more severely-exposed mothers were having their first children (abortions not being taken into account) later than the others. This could result from the exposure itself: perhaps it was that the somatic effects on the general health, or especially on the generative organs, resulted in these women marrying or conceiving later, or that chromosome aberrations in ovarian germ cells (especially likely due to neutrons: ABCC Report, p. 51) rendered the heavily-irradiated women temporarily sterile or caused them to conceive lethally malformed offspring which were eliminated early. Abortions were not considered in the report; further, it is admitted (p. 100) that many malformed infants could have

been disposed of early, before coming to the knowledge of the enquirers. The survey is concerned with major malformations diagnosed at birth; a sampling of the same population at the age of 9 months, however, showed that only 43 % of the malformations known at that time to exist, or to have existed, had been recognised at birth. This fact, coupled with the limited duration of the study, which encompassed only 6 years of one generation, means that only a small fraction were studied of the births among which a genetic-teratogenic effect of radiation might have been operative. Still, as we shall see later, the data on congenital malformations are consistent with the expected magnitude of effect.

*3. Stillbirths.* On the incidence of stillbirths, it is maternal exposure which seems to have the most marked effect (Table 2, Fig. 3). That the apparent effect of paternal exposure is not significant is due, doubtless, to an effect similar to that discussed in the preceding paragraph: namely, that heavy paternal exposure is associated with a lower mean parity. Because somatic effects on the father cannot be involved here, the whole effect must be genetic in nature. Azoöspermia due to roentgen rays has been known for a long time in rodents (ALBERS-SCHÖNBERG 1903, BERGONIÉ & TRIBONDEAU 1904) and in man (HUHNER 1935). In the Japanese seamen on board the Fifth Fukuryu Maru ("Lucky Dragon") who were sprinkled with thermonuclear dust near Bikini, in 1954, heavy exposure to radio-activity was followed by a long period of aspermia and infertility, lasting many months (TSUZUKI 1956, p. 1287). It is plausible that in Hiroshima and Nagasaki, following heavy irradiation of fathers, once recovery from temporary sterility had occurred, there were conceived a number of non-viable foetuses which were eliminated early. Such is the likely explanation for the fact that the mates of heavily-irradiated men bore their children later. The same effect is shown in the ABCC Report when, in a consideration of first-born infants only, it is found that the mothers of the stillborn infants were in the mean older than those of the liveborn, and this was especially marked in the offspring of heavily-exposed fathers. Because of this, the authors of the report disclaim the well-marked correlation, which they found, between stillbirth rates in first-born infants and paternal exposure, and go on to show that if the stillbirths are distributed into maternal age groups, the apparent effect of paternal exposure disappears.

This disappearance, however, is wholly due to the breaking of the sample into small groups. If, on the contrary, the same data are treated as a whole and corrected for maternal age, the highly significant relationship between paternal exposure and incidence of stillbirths in first births is conserved.

*4. Neo-natal deaths.* It appears from Table 2 that exposure of the father alone was associated with a higher neo-natal death rate, but not exposure

**Table 3**

*A ... y studies in the atomic bomb genetic survey. One study (Nagasaki) shows a significant effect of ir- ra ... ion, while the other (Hiroshima) does not. This disparity is seen to arise, in a large measure, from the difference in numbers involved, these being smaller in the Hiroshima survey.*

| | Total bodies | Abnormal bodies | $\chi^2$ | P |
|---|---|---|---|---|
| *Hiroshima (ABCC Report)* | | | | |
| ather only exposed ..... | 31 | 7 (22.6 %) | 0.40 | — |
| o parent exposed..... | 211 | 37 (17.5 %) | | |
| lother only exposed..... | 127 | 20 (15.8 %) | 0.16 | — |
| *Nagasaki (Hayashi)* | | | | |
| ather only exposed ..... | 80 | 18 (22.5 %) | 7.56 | < 0.01 |
| o parent exposed..... | 363 | 40 (11.0 %) | | |
| lother only exposed..... | 259 | 47 (18.1 %) | 6.49 | < 0.02 |
| t least one parent exposed within 2 km ..... | 72 | 20 (27.7 %) | 5.14 | < 0.03 |
| t least one parent exposed, but none nearer than 5 km ..... | 317 | 52 (16.4 %) | | |

(The unexposed population is excluded from the latter comparison.)

of the mother alone. Differences in anatomical shielding between male and female gonads may enter into consideration, but it is surprising that maternal somatic effects do not seem to play a rôle here. This, perhaps, may embolden us to discount the operation of any such somatic effect in the above-discussed apparent connexion between maternal irradiation and stillbirth, and increase the plausibility of the genetic nature of that effect.

5. *Total loss.* The data of the ABCC Report are given in such a way that offspring showing a given accident are removed from the group to be considered for the next accident; for instance, as inspection of the figures in Table 2 will show, the malformed children are removed from the number to be considered for stillbirth, etc. Thus, each child who might constitute an indicator of genetic factors is considered but once. This makes it permissible to summate the total effect, as seen in the incidence of malformations at birth, stillbirths and neo-natal deaths, and in the sex-ratio changes, as an index of the total reproductive failure. As seen in Table 2, the statistical evaluation of this summation yields a result which is highly significant in favour of the hypothesis of a genetic ill-effect of radiation. The notion of a wastage of offspring reflected by changes in the sex-ratio rests upon the assumption that the lowering of the sex-ratio in the mother-only-exposed group is due to the loss (through early absorption or abortion) of a certain number of males,

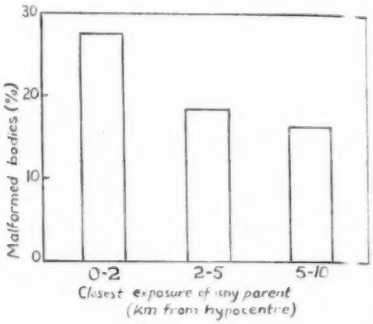


Fig. 4. Autopsy study of peri-natal deaths in Nagasaki (1949—1953). The proportion of seriously malformed bodies varies significantly with the distance from the detonation hypocentre. The unexposed population is excluded from this comparison. Based on data from HAYASHI (1956). See also Table 3.

and the raising of the sex-ratio in the father-only-exposed group is due to a loss of females (Fig. 1). Should one like to question the validity of this assumption, a total-loss value (excluding sex-ratio losses) can still be shown to be significant at the 3 % level. A summation of the data on paternal exposure alone (where somatic effects cannot be predicated) is also significant, whether or not the sex-ratio changes are taken into account (Table 2).

The ABCC Report also presents data of 406 autopsies done in Hiroshima, from May 1949 to December 1953, on infants stillborn or dying in the first 6 days of life; all were the product of at least 21 weeks' gestation. They represented 62 % of all deaths occurring in newborns registered in the study. The incidence of malformations in the autopsied bodies (including, this time, congenital cardiovascular disease) is distributed in groups on the basis of exposure or non-exposure of each or both parents. A difference appears in the incidence of anomalies, but it is not significant (Table 3-A). No information is given in the report on the frequency of various types of malformations found in the groups.

*Autopsy data in Nagasaki.* Independently of the ABCC survey, which we have reviewed in the preceding paragraphs, a series of autopsies on 884 foetuses and newborns was studied by I. Hayashi, Professor of Pathology in Nagasaki University, from September 1949 to December 1953. Since the data pertaining to this work are not available in any detail in non-Japanese literature, the report (HAYASHI 1956) is summarized here at some length. All anomalies found are listed with their distribution in the exposed and the non-exposed classes. Some common minor anomalies, such as accessory spleen, Marchand's accessory adrenal glands, reverse position of the sigmoid, mobile caecum, intrusion of the pancreas into the small intestine, and abnormal lobation of the lungs, are not taken into account. The malformations which were retained show a significant relation to both paternal and maternal exposure (Table 3 B) when the incidence of abnormal bodies in the father-only and the mother-

or -exposed groups is compared with that in the no-parent-exposed group. As to the types of malformations, diaphragmatic hernia and complex malformations of the heart and great vessels were more frequent in the exposed group. Some anomalies were seen only in the exposed group, including meningeal cyst (2 cases), atresia ani (3 cases) and atresia choledoci (2 cases). HAYASHI states that the histories of all mothers are being reviewed in order to rule out the operation of any factor, other than exposure, which could explain the differences found. This has been done for the cases of malformations of the heart and great vessels, which were found to bear no relation to 'sex, living birth, stillbirth, miscarriage, premature birth, normal birth, position in pregnancy, life duration after birth, season of conception, time of birth, mother's age, difference between father and mother, menstrual habit of mother, history of parents other than that of exposure, serological test, intermarriage, and numbers of pregnancy after exposure'. However, the mothers who bore offspring with cardiovascular anomalies, in 9 instances out of 12, had had abortion or miscarriage within the preceding year.

Here, then, are two reports on the autopsy incidence of congenital malformations in populations exposed to atomic bombs prior to conception. One survey, based on the findings in Hiroshima, fails to show an affect of radiation; in the other, from Nagasaki, a strong statistical suggestion is found in favour of the mutagenic effect of atomic radiation. It is true that the latter study, that of HAYASHI, deals with twice as many cases as the former. It is also possible that the different types of radiations (more gamma, fewer neutrons in Nagasaki) and the dissimilar climatic conditions in the two cities at the time of the explosions may account in part for the disparity. Nevertheless, the authors of the ABCC Report, who claim to have records on 363 autopsies in Nagasaki (which they fail to publish), question the validity of Hayashi's data and suggest, rather gratuitously, that a bias may create a spurious correlation. They claim, for instance, that the control figure (11.0 %) for the incidence of abnormal bodies from unirradiated parents is too low. However, it is a simple matter to compute from Hayashi's data the incidence of abnormal bodies in relation to distance from the hypocentre (Fig. 4); the comparison, which excludes the unexposed controls, yields a significant difference at the 3 % level (Table 3-B). This would seem to dispose of the doubts referred to above.

## Addendum

W. L. RUSSELL's (1960) latest results of chonic low-dose gamma irradiation of mouse spermatogonia support the prediction that no threshold dose, below which no genetic effect is incurred, would be demonstrable. While confirming the dependance, which his 1958 work had indicated, of the magnitude of effect on the dose rate, RUSSELL now fails to find a further reduction of the effect when the dose rate is decreased below 90 r per week; indeed, at 10 r/week the mutagenic effect per unit of radiation is no less marked than at 90 r/week. A dose of 8 r, given at the rate of 10 r/week, produces a highly significant increase in mutations.

## SUMMARY

Experimental, clinical and epidemiological data which have a bearing on the defini on of the genetic risk to man from ionizing radiations have been reviewed. Through a ew analysis of the official Report of the U. S. Commission of enquiry in Hiroshima and Nagas ki, based on a consideration of the irradiation of each parent separately, the probability of a harmful genetic effect in the first generation born to persons exposed to the atomic bo bs in 1945 is demonstrated. The total effect, as manifested by altered sex-ratio, congei tal malformations and peri-natal mortality, is significant even when one considers only he exposure of fathers, in whom somatic changes cannot contribute to the observed effect. Aut osy evidence for the teratogenic effect of atomic radiations is also reviewed. The significanc of these data in relation to the expected mutagenic effect on man of actual and possible loac of ionizing radiations will be discussed in Part II.

## ZUSAMMENFASSUNG

Experimentelle, klinische und epidemiologische Daten, die mit der Definition des g ne-tischen Risikos des Menschen gegenüber jonisierender Strahlung im Zusammenhang st ien können, sind nachgeprüft worden. Durch eine neue Analyse des offiziellen Rapportes der amerikanischen Untersuchungskommission in Hiroshima und Nagasaki, welche sich auf die Betrachtung der Bestrahlung von Eltern separat gründen, wird die Möglichkeit eines sch id-lichen genetischen Effektes in der ersten Generation von Kindern nachgewiesen, die von Personen geboren wurden, welche der Atombombenbestrahlung 1945 ausgesetzt waren. Der totale Effekt, manifestiert durch eine veränderte Geschlechtsratio, kongenitale Missbildun gen und perinatale Tödlichkeit, ist signifikant, auch wenn man nur die Exponierung der V ter in Betracht zieht, bei denen somatische Veränderungen nicht zu dem beobachteten Effekt beitragen können. Der anatomische Nachweis des teratogenen Effektes der Atomstrahlung ist ebenfalls nachgeprüft worden. Die Signifikanz dieser Daten in Beziehung zu dem erwarteten mutagenen Effekt beim Menschen durch aktuelle oder mögliche Strahlenbelastung wird in Teil II diskutiert.

## RÉSUMÉ

Revue critique des données expérimentales, cliniques et épidémiologiques permettant de préciser le risque génétique pour l'homme des rayonnements ionisants. Une nouvelle analyse du Rapport officiel de la commission américaine d'enquête à Hiroshima et Nagasaki, où l'on considère séparément l'irradiation de chaque parent, permet de démontrer la probabilité d'un effet génétique défavorable à la première génération issue de parents exposés aux bombes atomiques de 1945. L'effet global, qui se manifeste dans le rapport des sexes, les malfaçons congénitales et la mortalité para-natale, demeure significatif même si l'on ne considère que l'irradiation des pères, chez qui on ne saurait invoquer d'altération somatique. On rapporte enfin les preuves anatomiques de l'effet tératogène des rayonnements atomiques. La signification de ces données quant à l'effet mutagène attendu des doses actuelles et possibles de rayonnements ionisants fera l'objet d'une deuxième partie de cette étude.


## REFERENCES

For a complete bibliography see Part II which will appear in next number of Act radiologica, 56 (1961), 145.